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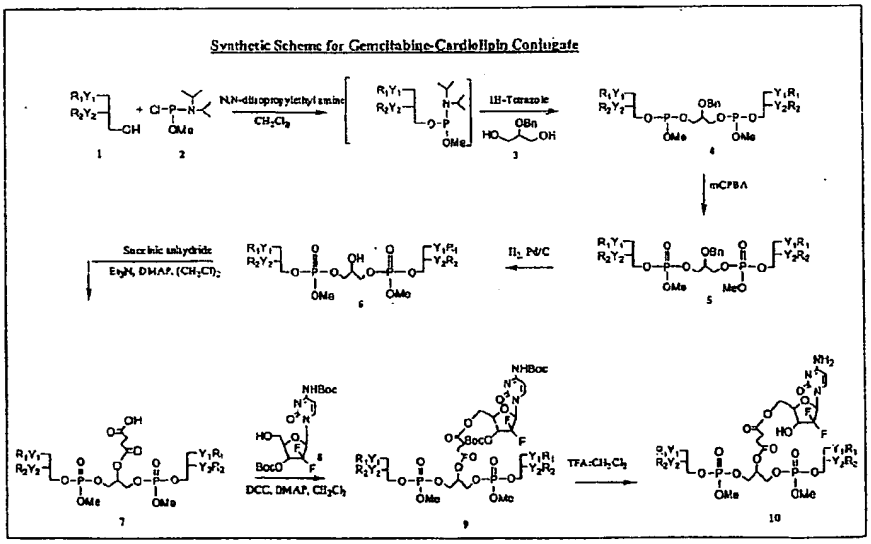
(54) Title: NUCLEOSIDE-LIPID CONJUGATES, THEIR METHOD OF PREPARATION AND USES THEREOF



(57) Abstract: The invention provides methods for synthesizing nucleoside-lipid conjugates having varying fatty acid and alkyl chain lengths with or without unsaturation and their use in the treatment of cancer and viral diseases. More particularly, the invention provides methods for preparing gemcitabine-cardiolipin conjugates, and analogues thereof, cytarabine-cardiolipin conjugates, and analogues thereof. Additionally, the methods of the invention comprise administering a compound of invention as prodrug or a pharmaceutical preparation to combat mammalian diseases, preferably cancer, viral infections and bone disorders.

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NUCLEOSIDE-LIPID CONJUGATES, THEIR METHOD OF PREPARATION AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICTIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 60/405378 filed on September 2, 2004, the disclosure of which is incorporated herein.

FIELD OF THE INVENTION

[0002] This invention is related to novel nucleoside-lipid conjugates, the methods of preparing them and the compositions that contain them. The novel nucleoside-lipid conjugates comprise nucleosides and nucleoside analogues conjugated with phospholipids such as cardiolipin and cardiolipin analogues. In addition, the invention relates to the methods of preparing gemcitabine-cardiolipin conjugates, cytarabine-cardiolipin conjugates and analogues thereof. The invention also relates to the use of such nucleoside-lipid conjugates for the treatment of mammalian diseases, such as cancer and viral infections.

BACKGROUND OF THE INVENTION

[0003] Nucleoside analogues are compounds which mimic naturally occurring nucleosides by participating in nucleic acid metabolism. To date, several nucleoside analogues have been developed for the treatment of cancer and viral infections. Some examples include dideoxynucleoside analogues, such as 3'-Azido-3'deoxythymidine (AZT) (Mitsuya et al. *Proc. Natl. Acad. Sci. U.S.A.* 1985, *82*, 7096-7100), dideoxyinsosine (ddI), dideoxycytidine (ddC) (Mitsuya and Border. *Proc. Natl. Acad. Sci. U.S.A.* 1986, *83*, 1911-1915), 2'3'-didehydro-2'dideoxythymidine (d4T) and 3'-azido-2',3'-dideoxyuridine (AZddU) (Balzarini *et al. Mol. Pharmacol.* 1987, *32*, 162-167). Another example is cytarabine (1-β-D-arabinofuranosylcytosine, ara-C) which is a pyrimidine nucleoside analogue used for the treatment of hematological malignancies, such as acute myelogenous leukemia and non-Hodgkin's lymphoma. Another example is fludarabine (9-β-D-arabinofuranosyl-2-fluoroadenine) which is a purine nucleoside analog used to treat low-grade lymphomas and chronic lymphocytic leukemia (Chun *et al., J. Clin. Oncol.*, 1991, *9*, 175-188). Cladribine (2-chloro-2'-deoxyadenosine, 2-CdA) is another nucleoside analogue that has been shown to be active in low-grade lymphomas and chronic lymphocytic leukemia (Damaraju *et al, Oncogene*, 2003, *22*, 7524-7536). Another example is capecitabine (5'-deoxy-5-N-[(pentoxy)carbonyl]cytidine) which is a pyrimidine nucleoside analogue and a prodrug of 5'-fluorouracil. Capecitabine, after oral administration, is metabolized by carboxyesterases to 5'-deoxy-5-fluorocytidine which, in turn, is deaminated by cytidine deaminase to 5'deoxy-5-fluorouridine. Another example is gemcitabine (2',2'-difluorodeoxyribofuranosylcytosine) which is a difluorinated analogue of deoxycytidine currently marketed

as Gemzar®. Gemcitabine is used for the treatment of non-small cell lung and pancreatic cancer. It is a potent antitumor agent in murine and human xenograft solid tumor models (Myhren *et al.*US 6,384,019 B1, Johnson, P.G. *et al.*, *Cancer Chromatography and Biological Response Modifiers*, Annual 16, Chap. 1, ed. Pinedo *et al.* 1996).

[0004] The limitations of these drugs include anticancer drug resistance, inadequate delivery of drugs to the brain and central nervous system (CNS), inadequate uptake of drugs by lymphoid and hematopoietic tissues, toxicity, oral bioavailability, short-drug half-life, and extracellular drug metabolism (Kucera *et.al.* US 2002/0082242 A1).

[0005] Methods, that have been employed to circumvent these problems, include the development of lipid-nucleosides conjugates, prodrugs, and liposome preparations. A series of anti-HIV nucleosides conjugates of ether (1-*O*-alkyl) and thioether (1-*S*-alkyl) lipids, linked by a pyrophosphate diester (diphosphate) bond, has been synthesized as micelle-forming prodrugs of the nucleosides to improve their therapeutic efficacy. These include AZT-5'-diphosphate-*rac*-1-*S*-octadecyl-2-*O*-palmitoyl-1-thioglycerol, 3'-azido-2',3'-dideoxyuridine-5'-diphosphate-*rac*-1-*S*-octadecyl-2-*O*-palmitoyl-1-thioglycerol, 2',3'-dideoxycytidine-5'diphosphate-rac-1-*S*-octadecyl-2-*O*-palmitoyl-1-thioglycerol and AZT 5'-diphosphate-rac-1-*O*-tetradecyl-2-*O*-palmitoylglycerol (Hong *et al J. Med. Chem.*, 1996, *39*, 1771-1777; Hong, *et al.* US Patent 5,484,911); Hong, *et. al. J. Lipid Mediators Cell Signalling,*(1994), *10,* 159-161. In addition, nucleoside phosphonate analogues, covalently linked to a substituted or unsubstituted alkylglycerol, alkylpropanediol, alkylethanediol such as Adefovir hexadecyloxypropyl ester, AZT-phosphonate hexadecyloxypropyl ester, 1-*O*-hexadecyloxypropane-3-Alendronate, Cidofovir hexadecyloxypropyl, Cidofir octadecyloxypropyl, Cidofir octadecyloxyethyl ester etc., were developed to target viral and cancer diseases (Hostetler *et al* US Patent No: 6,716,825 B2). Furthermore, the synthesis of gemcitabine derivatives such as elaidic acid (5')-gemcitabine ester and elaidic acid (N^4)-gemcitabine amide (Myhren *et al.* US 6,384,019 B1) have improved the half-life of gemcitabine.

[0006] Most of the nucleoside-lipid conjugates exhibited improved anticancer and antiviral efficacies compared to their respective parent compounds. Among them, the most effective conjugates includes ara-CDP-*rac*-1-*S*-tetradecyl-2-*O*-palmitoyl-1-thioglycerol, ara-CDP-*rac*-1-*S*-octadecyl-2-*O*-palmitoyl-1-thioglycerol, ara-CDP-*rac*-1-*S*-octadecyl-2-*O*-linoleoyl-1-thioglycerol, AZTDP-*rac*-1-*S*-octadecyl-2-*O*-palmitoyl-1-thioglycerol and 3'-azido-2',3'-dideoxyuridine (AZddU) analogue (Hong, *et al, J. Lipid Mediators Cell Signalling,* 1994, *10*, 159-161).

BRIEF SUMMARY OF THE INVENTION

[0007] The invention provides methods for synthesizing nucleoside and nucleoside analogues conjugated with cardiolipin and cardiolipin analogues such as gemcitabine-cardiolipin conjugates, cytarabine-cardiolipin conjugates and analogues thereof. Additionally, the invention provides for a method of administering such compositions as a prodrug or administering a pharmaceutical composition comprising the invention as a prodrug to in order to combat mammalian diseases.

BRIEF DESCRIPTION OF THE INVENTION

[0008] Figure 1 depicts the general structure for the 5-membered cyclic sugars.

[0009] Figure 2 depicts a scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0010] Figure 3 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0011] Figure 4 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0012] Figure 5 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0013] Figure 6 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0014] Figure 7 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0015] Figure 8 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0016] Figure 9 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0017] Figure 10 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0018] Figure 11 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0019] Figure 12 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0020] Figure 13 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0021] Figure 14 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0022] Figure 15 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0023] Figure 16 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0024]

[0025] Figure 17 depicts an alternate scheme for synthesizing gemcitabine-cardiolipin conjugates.

[0026] Figure 18 depicts the structure for a gemcitabine-cardiolipin conjugate.

[0027] Figure 19(a) depicts gemcitabine-cardiolipin conjugate and gemcitabine induced dose-dependent cytotoxicity in cancer cells.

[0028] Figure 19(b) depicts gemcitabine-cardiolipin conjugate and gemcitabine induced time-dependent cytotoxicity in cancer cells.

[0029] Figure 20 depicts gemcitabine-cardiolipin conjugate and gemcitabine induced caspase 3/7 expression.

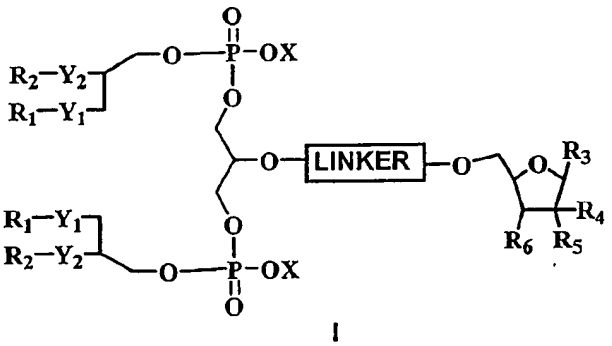
[0030] Figure 21 depicts the effect of dipyridamole on gemcitabine-cardiolipin conjugate-induced cytotoxicity.

[0031] Figure 22 depicts the percent survival rate after administration of gemcitabine-cardiolipin conjugates or gemcitabine.

[0032] Figure 23 depicts the effect on tumor growth after administration of gemcitabine-cardiolipin conjugates or gemcitabine.

DETAILED DESCRIPTION OF THE INVENTION

[0033] The present invention describes methods for synthesizing nucleoside-cardiolipin conjugates having general formula I.



I

Wherein Y_1 and Y_2 are the same or different and are –O-C(O)-, -O-, -S-, -NH-C(O)- or the like;

R_1 and R_2 are the same or different and are selected from a group consisting of H, saturated alkyl group and unsaturated alkyl group;

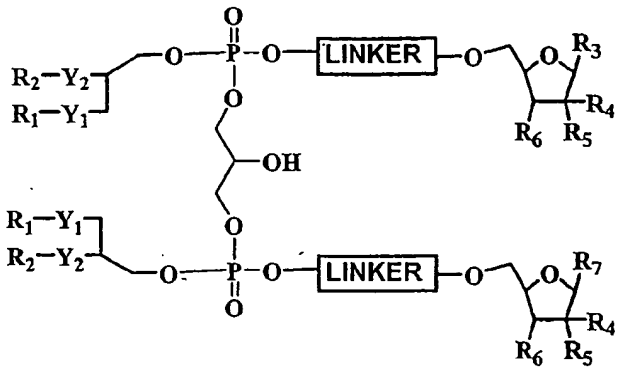
X is selected from a group consisting of H, alkyl group ranging from C_1 to C_{10}, and a cation (preferably a non-toxic cation such as ammonium, sodium, potassium, calcium and barium);

R_3 is selected from a group consisting of cytosine, guanine, adenine, thymine, uracil, inosine, hypoxanthine and xanthine, wherein R_3 is optionally substituted with one, two, three or four substituents selected from a group consisting of halo, nitro, alkyl, alkenyl, alkoxy, aryl, triflurormethyl, and $N(R^a)(R^b)$ wherein R^a and R^b are independently selected from the group consisting of H and (C_1-C_8) alkyl;

R_4 and R_5 are the same or different and are selected from a group consisting of a halo group (H, F, Cl, Br, I), nitro, hydroxyl, substituted alkyl, an alkyl group (C_1-C_{15}) and an alkoxy group (such as methoxy, ethoxy, propoxy, butoxy, and polyalkoxy); and

R_6 is selected from a group consisting of OH, azido group, amino group, substituted amino, alkyl group, and a halo group (H, F, Cl, Br, I).

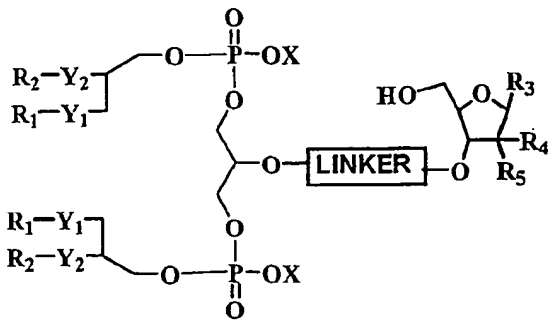
[0034] The present invention also describes methods for the preparation of nucleoside-cardiolipin conjugates having general formula II.



II

Wherein Y_1 and Y_2 are the same or different and are –O-C(O)-, -O-, -S-, -NH-C(O)- or the like;
R_1 and R_2 are the same or different and are selected from a group consisting of hydrogen, saturated alkyl group and unsaturated alkyl group;
R_3 and R_7 are the same or different and are selected from a group consisting of cytosine, guanine, adenine, thymine, uracil, inosine, hypoxanthine and xanthine, wherein R_3 and/or R_7 are optionally substituted with one, two, three or four substituents selected from a group consisting of halo, nitro, alkyl, alkenyl, alkoxy, aryl, triflurormethyl, and $N(R^a)(R^b)$ wherein R^a and R^b are independently selected from the group consisting of H and (C_1-C_8) alkyl;
R_4 and R_5 are the same or different and are selected from a group consisting of a halo group (H, F, Cl, Br, I), nitro, hydroxyl, substituted alkyl, alkyl group (C_1-C_{15}), alkoxy group (such as methoxy, ethoxy, propoxy, butoxy, polyalkoxy group); and
R_6 is selected from a group consisting of OH, azido group, amino group, substituted amino, alkyl group, and a halo group (H, F, Cl, Br, I).

[0035] The present invention also describes methods for the preparation of nucleoside-cardiolipin conjugates having general formula **III.**



III

Wherein Y_1 and Y_2 are the same or different and are –O-C(O)-, -O-, -S-, -NH-C(O)- or the like;

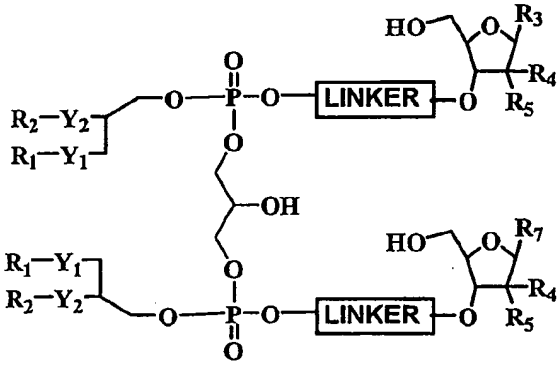
R_1 and R_2 are the same or different and are selected from a group consisting of H, saturated and unsaturated alkyl group;

X is selected from a group consisting of H, alkyl group ranging from C_1 to C_{10}, and a cation (preferably a non-toxic cation such as ammonium, sodium, potassium, calcium and barium);

R_3 is selected from a group consisting of cytosine, guanine, adenine, thymine, uracil, inosine, hypoxanthine and xanthine, wherein R_3 is optionally substituted with one, two, three or four substituents selected from a group consisting of halo, nitro, alkyl, alkenyl, alkoxy, aryl, trifluoromethyl, and $N(R^a)(R^b)$ wherein R^a and R^b are independently selected from a group consisting of H and (C_1-C_8) alkyl groups;

R_4 and R_5 are the same or different and are selected from a group consisting of a halo group (H, F, Cl, Br, I), nitro, hydroxyl, substituted alkyl, alkyl group (C_1-C_{15}), alkoxy group (such as methoxy, ethoxy, propoxy, butoxy and polyalkoxy).

[0036] The present invention also describes methods for the preparation of nucleoside-cardiolipin conjugates having general formula IV.



IV

Wherein Y_1 and Y_2 are the same or different and are –O-C(O)-, -O-, -S-, -NH-C(O)- or the like;

R_1 and R_2 are the same or different and are selected from a group consisting of H, saturated alkyl group and unsaturated alkyl group;
R_3 and R_7 are the same or different and are selected from a group consisting of cytosine, guanine, adenine, thymine, uracil, inosine, hypoxanthine and xanthine, wherein R_3 and/or R_7 is optionally substituted with one, two, three or four substituents selected from a group consisting of halo, nitro, alkyl, alkenyl, alkoxy, aryl, trifluoromethyl, and $N(R^a)(R^b)$ wherein R^a and R^b are independently selected from a group consisting of H and (C_1-C_8) alkyl; and
R_4 and R_5 are the same or different and are selected from a group consisting of a halo group (H, F, Cl, Br, I), nitro, hydroxyl, substituted alkyl, alkyl group (C_1-C_{15}), alkoxy group (such as methoxy, ethoxy, propoxy, butoxy and polyalkoxy).

[0037] The term "linker" is defined herein as a group or chain containing one or more functional group for covalent binding with the lipid carrier and biologically active nucleoside. Preferred embodiments comprise a linker having at least two functional groups, wherein the linker has a first end and a second end and wherein the lipid is attached to the first end of the linker through a first linker functional group and the nucleoside is attached the second end of the linker through a second linker functional group. These groups can be designated either as weak or strong, based on the stability of the covalent bond which the linker functional group will form between the linker and either the lipid carrier or the biologically-active nucleoside. The weak functionalities include, but are not limited to, phosphoramidite, phosphoesters (such as phosphodiester, phosphotriester and phosphonate), carbonate, amide, carboxyl-phosphoryl anhydride, ester and thioester. The strong functionalities include, but are not limited to, ether, thioether, amine, amide, and ester. The use of a strong linker functional group between the linker and the nucleoside will tend to decrease the rate at which the compound will be released *in vivo*, whereas the use of a weak linker functional group between the linker and the nucleoside may act to facilitate *in vivo* release of the compound. In preferred embodiments, each of the first and second functional linker groups is a hydroxyl group, a primary or secondary amino group, phosphate group or substituted derivative thereof, a carboxylic acid, carbonate, carbamate or carbonyl group. The "linker" herein also comprises, in addition to the functional groups at either end, $(CH_2)_n$ groups (where n = 0-20) in the center optionally substituted with functional groups, such as alkyl, alkoxy, hydroxyl, carbonyl, carboxyl, carbamate, aldehyde, amino, halo, polyalkoxy, PEG groups, phosphate, phosphonate and pyrophosphate groups.

[0038] The term "lipid" herein includes cardiolipin and cardiolipin derivatives or analogues having varying fatty acid and or alkyl chain with or without unsaturation.

[0039] The term "prodrug" is defined as a pharmacologically inactive compound that is converted into an active agent by metabolic transformation. The objective is to chemically modify the antitumor agent (into its prodrug) in order to render it temporarily inactive. *In vivo*, via the action of enzyme(s), this

prodrug then decomposes thereby liberating the active principle. In most cases, a judiciously selected chemical group is bound covalently to the active principle. This group will often govern the solubility of the prodrug, its stability, the rate at which it liberates the active principle and the particular enzyme(s) required for its transformation (Malet-Martino *et al. Curr. Med. Chem.-Anti-Cancer Agents*, 2002, *2*, 267-310)

[0040] The term "therapeutic agent" herein means any compound or composition, which upon entering a mammalian cell, is capable of contributing to the alleviation or treatment of a mammalian disease. The mammalian cell can be any type of mammalian cell, including both cancerous and non-cancerous cells. The cancer can be any type of cancer in a mammal. By way of example and not by limitation, "therapeutic agents" include small organic molecules, peptides, nucleoside analogues, anticancer agents, antiviral agents, ribozymes, protease inhibitors, polymerase inhibitors, reverse transcriptase inhibitors, antisense oligonucleotides and other drugs. As used herein, the term "anticancer agent" means a therapeutic agent capable of exhibiting efficacy at combating a cancer in a mammal or in a mammalian cell, or any compound which is capable of being converted intracellularly to a compound which is capable of exhibiting efficacy at combating a cancer in a mammal or in a mammalian cell. As used herein, the term, "combating a cancer" means any one or more of the following: to increase survival of a mammal, to arrest or decrease tumor size in a mammal, or to increase the time period of remission of cancer regrowth in a mammal.

[0041] The term "alkyl" encompasses saturated or unsaturated straight chain and branched-chain hydrocarbon moieties. The term "substituted alkyl" comprises alkyl groups further bearing one or more substituents selected from hydroxyl, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), cycloalkyl, substituted cycloalkyl, halogen, cyano, nitro, amino, amido, imino, thio, -C(O)H, acyl, oxyacyl, carboxyl and the like.

[0042] In a preferred embodiment, for formulae I-IV, the five-membered cyclic sugar is ribofuranose, arabinofuranose, deoxyribofuranose or xylofuranose. The nomenclature is based on the specific orientation or absence of the hydroxyl groups at C2'and C3' position and the attachment of the heterocylic base at C1'(Figure 1).

[0043] It must be noted that the nucleoside moiety, in the formulae above (I-IV), does not indicate the stereochemistry of the compounds of the present invention, and the stereochemistry is not a critical aspect of the invention. Accordingly, it will be understood that the present invention refers to a nucleoside-lipid conjugate of all possible stereochemical orientations, while recognizing that certain stereochemical species will be found to be more effective than other orientations. For example, it has been found that nucleosides with β configuration (attachment of base to the 5-membered cyclic sugar at C1') are more

efficacious than the α-nucleosides. The β-nucleosides are therefore preferred compounds for making the present nucleoside-lipid conjugates.

[0044] In a preferred embodiment, for formulae I-IV, R_1 and R_2 are the same or different and are selected from a group comprising C_1-C_{34} saturated and/or unsaturated alkyl group, more preferably between C_1 and C_{24} carbon atoms.

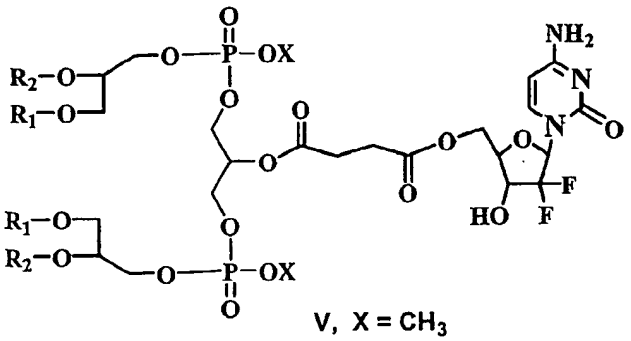
[0045] In a preferred embodiment, R_3 is cytosine, guanine, adenine, thymine, uracil, inosine, hypoxanthine, xanthine, wherein R_3 is optionally substituted with one, two, three or four substituents selected from a group consisting of halo, nitro, alkyl, alkenyl, alkoxy, aryl, trifluoromethyl, and $N(R^a)(R^b)$, wherein R^a and R^b are independently selected from a group consisting of H and (C_1-C_8) alkyl.

[0046] In a preferred embodiment, R_4 and R_5 are the same or different and are selected from a group consisting of a halo (H, F, Cl, Br, I), nitro, hydroxyl, alkyl, substituted alkyl, and alkoxy group (such as methoxy, ethoxy, propoxy, butoxy group).

[0047] In a preferred embodiment, R_6 is preferably a hydroxyl group, azido, amino, substituted amino, or halo (H, F, Cl, Br, I) group.

[0048] In a preferred embodiment, the linker comprises an alkyl, substituted alkyl, dicarbonyl alkyl (for example, succinimidoyl group), alkoxy, polyalkoxy, PEGylated (PEG) group, phosphate, phosphonate, diphosphate, triphosphate, phosphodiester, phosphotriester, phosphoramidite, a peptide, dipeptide, polypeptide and the like.

[0049] In a more preferred embodiment, the compound, according to general formula I, is a gemcitabine-cardiolipin conjugate having structures V and VI, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, R_6 is hydroxyl group, X is methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, the linker is succinimidoyl group and

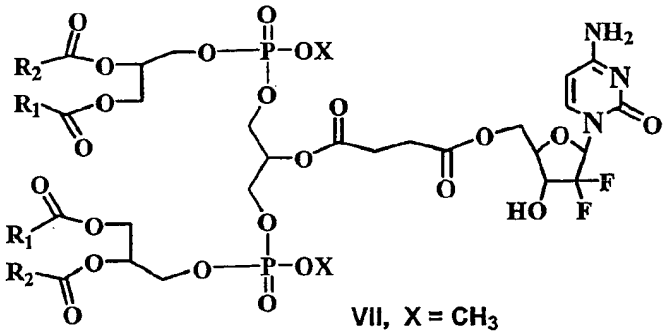


V, X = CH_3

VI, X = NH4

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0050] In a more preferred embodiment, the compound, according to general formula **I**, is a gemcitabine-cardiolipin conjugate having structures **VII** and **VIII**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, R_6 is hydroxyl group, X is methyl or ammonium, Y_1, Y_2 are -O-C(O)-, the linker is a succinimidoyl group, and

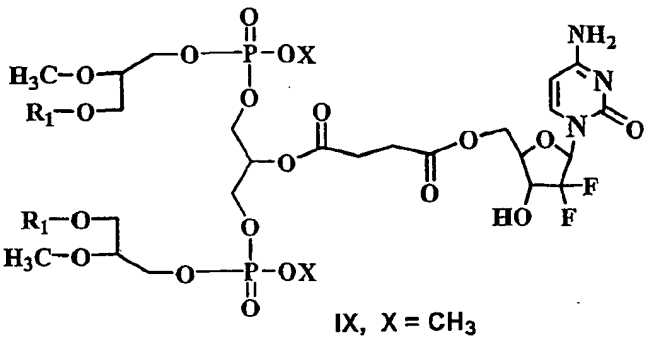


VII, X = CH_3

VIII, X = NH4

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0051] In a more preferred embodiment, the compound, according to general formula I,is a gemcitabine-cardiolipin conjugate having structures **IX** and **X**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, R_6 is hydroxyl group, X is methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, the linker is a succinimidoyl group, and



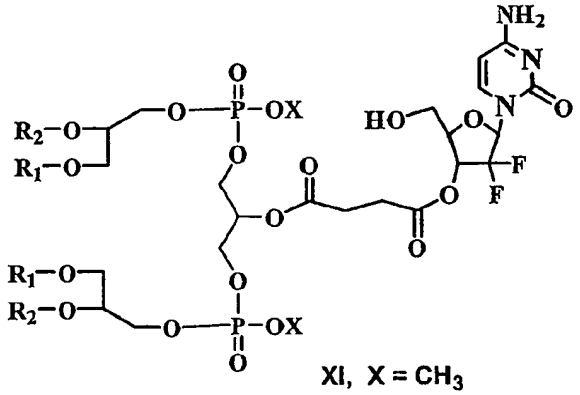
IX, X = CH_3

⊕

X, X = NH4

R_1 is H, C_1-C_{34} saturated or unsaturated alkyl groups, and R_2 is a methyl group.

[0052] In a more preferred embodiment, the compound, according to general formula **III**, is a gemcitabine-cardiolipin conjugate having structures **XI** and **XII**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, X is methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, the linker is succinimidoyl group, and



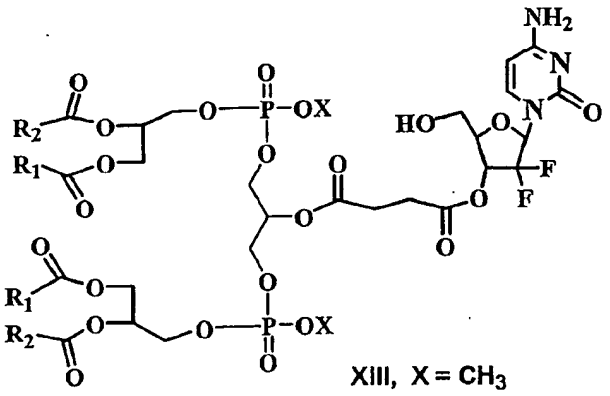
XI, X = CH_3

⊕

XII, X = NH4

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0053] In a more preferred embodiment, the compound, according to general formula **III**, is a gemcitabine-cardiolipin conjugate having structures **XIII** and **XIV**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, X is methyl or ammonium, Y_1, Y_2 are –O-(CO)- groups, the linker is a succinimidoyl group, and



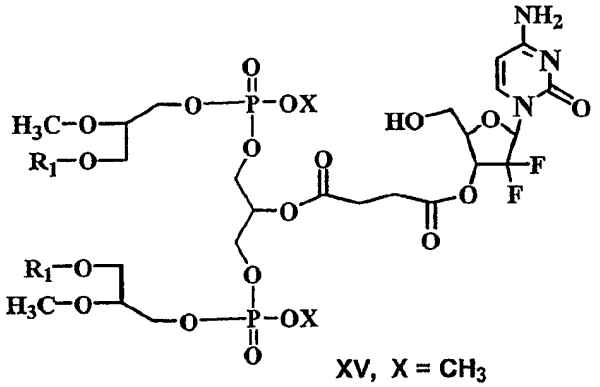
XIII, X = CH_3

⊕

X1V, X = NH4

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0054] In a more preferred embodiment, the compound, according to general formula **III**, is a gemcitabine-cardiolipin conjugate having structures **XV** and **XVI**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, X is methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, the linker is succinimidoyl group,



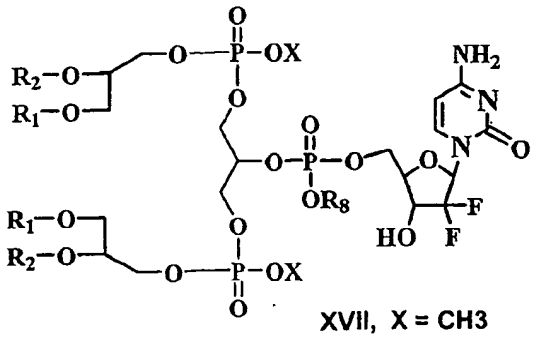
XV, X = CH_3

⊕

XVI, X = NH4

R_1 is H, C_1-C_{34} saturated or unsaturated alkyl groups and R_2 is a methyl group.

[0055] In a more preferred embodiment, the compound, according to general formula **I**, is a gemcitabine-cardiolipin conjugate having structures **XVII** and **XVIII**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, R_6 is hydroxyl group, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, and the linker is a phosphodiester (R_8 is ammonium) or phosphotriester (R_8 is a methyl) and

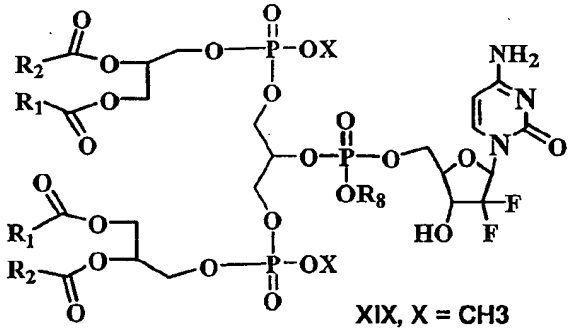


XVII, X = CH3

XVIII, X = $\overset{\oplus}{N}H4$

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0056] In a more preferred embodiment, the compound, according to general formula **I**, is a gemcitabine-cardiolipin conjugate having structures **XIX** and **XX**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, R_6 is hydroxyl group, X and R_8 are methyl or ammonium, Y_1, Y_2 are –O-(CO)- groups, and the linker is a phosphodiester (R_8 is ammonium) or phosphotriester (R_8 is a methyl), and

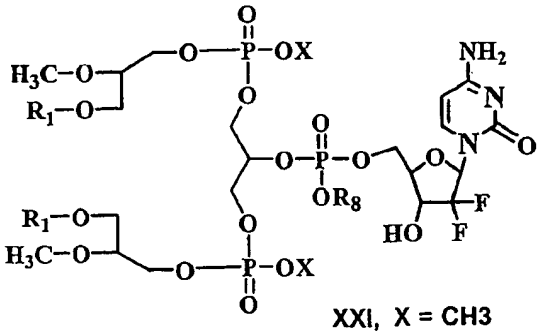


XIX, X = CH3

XX, X = $\overset{\oplus}{N}H4$

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0057] In a more preferred embodiment, the compound, according to general formula **I**, is a gemcitabine-cardiolipin conjugate having structures **XXI** and **XXII**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, R_6 is hydroxyl group, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, the linker is a phosphodiester (R_8 is ammonium) or phosphotriester (R_8 is a methyl),

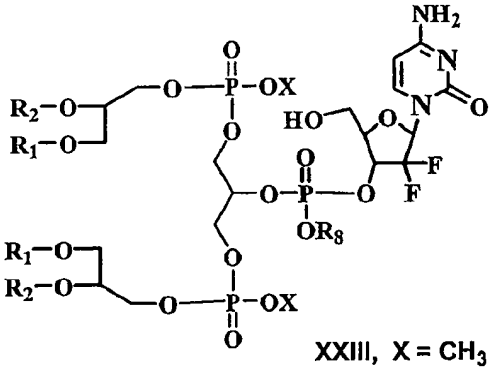


XXI, X = CH3

⊕
XXII, X = NH4

R_1 is H, C_1-C_{34} saturated or unsaturated alkyl groups, and R_2 is methyl group.

[0058] In a more preferred embodiment, the compound, according to general formula III, is a gemcitabine-cardiolipin conjugate having structures XXIII and XXIV, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, the linker is a phosphodiester (R_8 is ammonium) or phosphotriester (R_8 is a methyl),

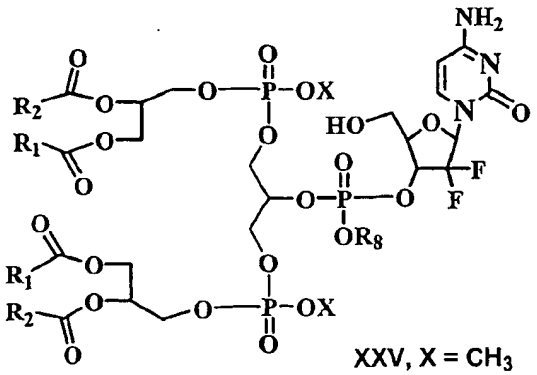


XXIII, X = CH_3

⊕
XXIV, X = NH4

and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0059] In a more preferred embodiment, the compound, according to general formula III, is a gemcitabine-cardiolipin conjugate having structures XXV and XXVI, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, X and R_8 are methyl or ammonium, Y_1, Y_2 are –O-(CO)- groups, and the linker is a phosphodiester (R_8 is ammonium) or phosphotriester (R_8 is a methyl),



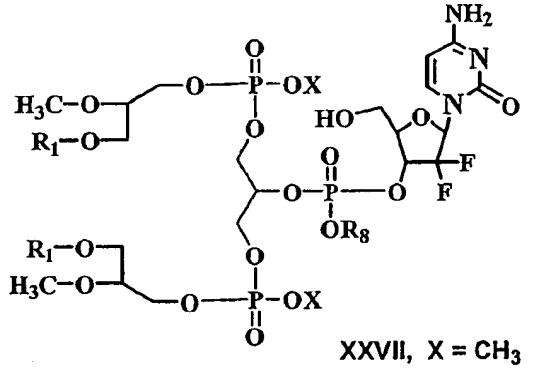
XXV, X = CH_3

$\oplus$

XXVI, X = NH4

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0060] In a more preferred embodiment, the compound, according to general formula **III**, is a gemcitabine-cardiolipin conjugate having structures **XXVII** and **XXVIII**, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, the linker is a phosphodiester (R_8 is ammonium) or phosphotriester (R_8 is a methyl),



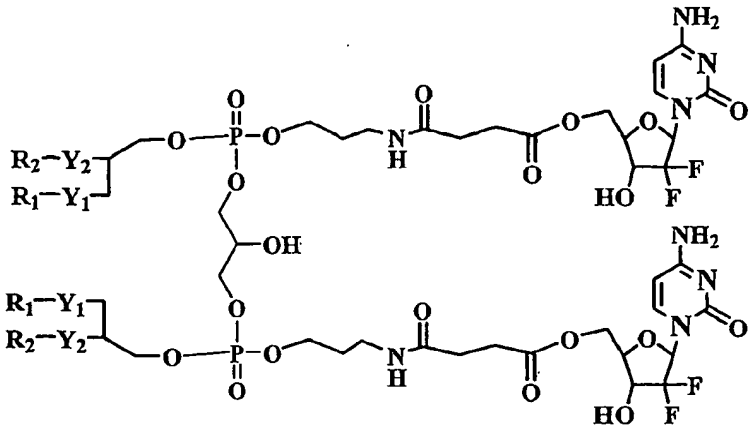
XXVII, X = CH_3

$\oplus$

XXVIII, X = NH4

R_1 is H, C_1-C_{34} is saturated or unsaturated alkyl groups, and R_2 is a methyl group.

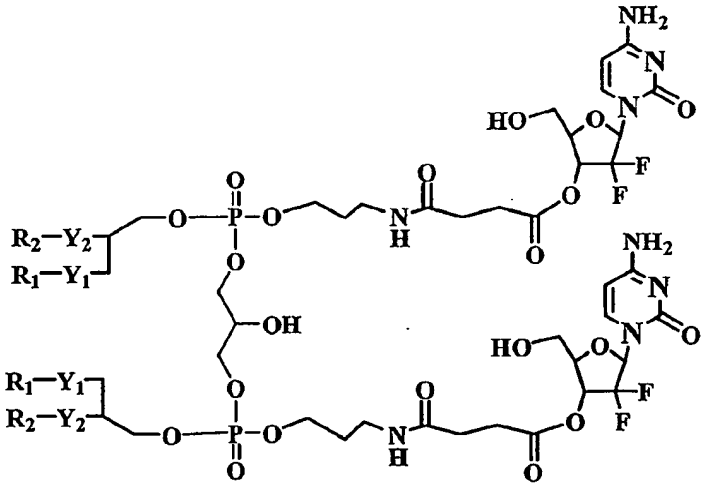
[0061] In a more preferred embodiment, the compound, according to general formula **II**, is a gemcitabine-cardiolipin conjugate having structure **XXIX**, wherein R_3 and R_7 are cytosine, R_4 and R_5 are fluoro groups, R_6 is hydroxyl, Y_1, Y_2 are oxo (-O-) or –O-C(O)- groups, the linker is –$CH_2CH_2CH_2NHCOCH_2CH_2C(O)$-group, and



XXIX

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups

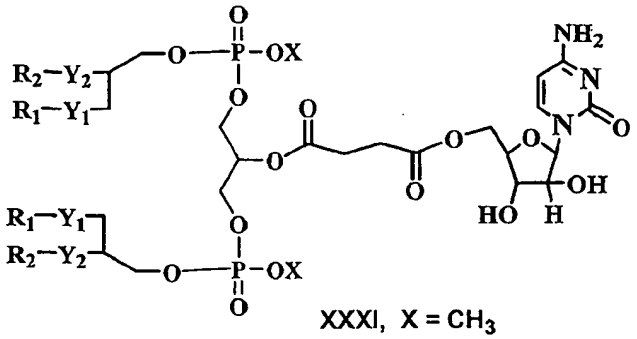
[0062] In a more preferred embodiment, the compound, according to general formula IV, is a gemcitabine-cardiolipin conjugate having structure XXX, wherein R_3 and R_7 are cytosine, R_4 and R_5 are fluoro groups, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is $-CH_2CH_2CH_2NHCOCH_2CH_2C(O)$- group,



XXX

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups

[0063] In a more preferred embodiment, the compound, according to general formula I, is a cytarabine-cardiolipin conjugate having structures XXXI and XXXII, wherein R_3 is cytosine, R_4 and R_6 are hydroxyl, R_5 is hydrogen, X is methyl or ammonium, Y_1, Y_2 are oxo (-O-) or –O-C(O)- groups, and the linker is succinimidoyl group,



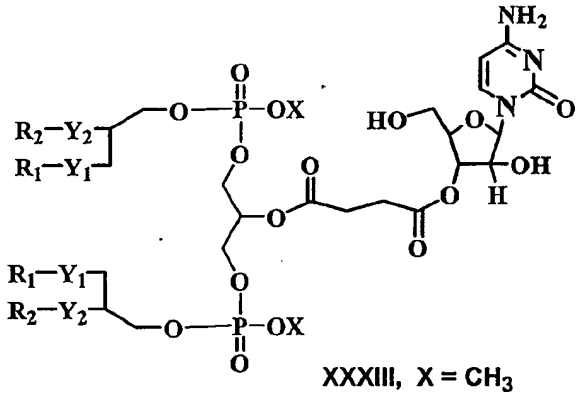
XXXI, X = CH_3

$\oplus$

XXXII, X = NH4

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0064] In a more preferred embodiment, the compound, according to general formula III, is cytarabine-cardiolipin conjugate having structures XXXIII and XXXIV, wherein R_3 is cytosine, R_4 is hydroxyl, R_5 is hydrogen, X is methyl or ammonium, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is succinimidoyl group, and



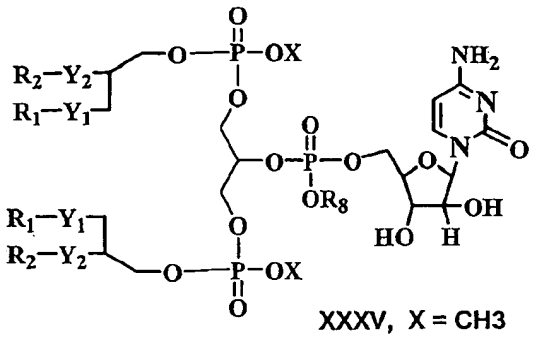
XXXIII, X = CH_3

$\oplus$

XXXIV, X = NH4

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

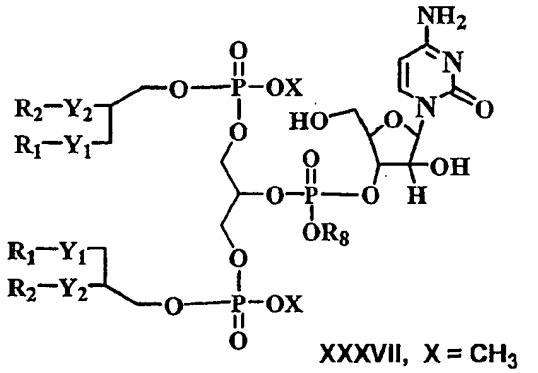
[0065] In a more preferred embodiment, the compound, according to general formula I, is a cytarabine-cardiolipin conjugate having structures XXXV and XXXVI, wherein R_3 is cytosine, R_4 and R_6 are hydroxyl, R_5 is hydrogen, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) or –O-C(O)- groups, the linker is a phosphodiester (R_8 is ammonium) or phosphotriester (R_8 is a methyl), and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.



XXXV, X = CH3

XXXVI, X = $\overset{\oplus}{N}$H4

[0066] In a more preferred embodiment, the compound, according to general formula III, is a cytarabine-cardiolipin conjugate having structures XXXVII and XXXVIII, wherein R_3 is cytosine, R_4 is hydroxyl and R_5 is hydrogen, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is a phosphodiester (R_8 is ammonium) or phosphotriester (R_8 is a methyl), and



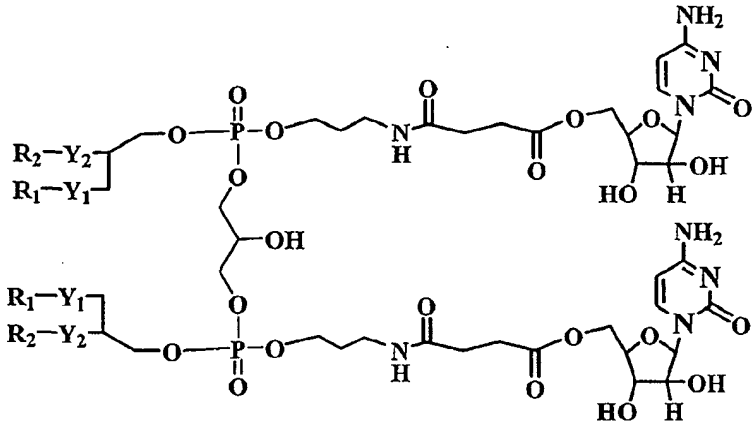
XXXVII, X = CH_3

XXXVIII, X = $\overset{\oplus}{N}$H4

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0067] In a more preferred embodiment, the compound, according to general formula II, is a cytarabine-cardiolipin conjugate having structure XXXIX, wherein R_3 and R_7 are cytosine, R_4 and R_6 are

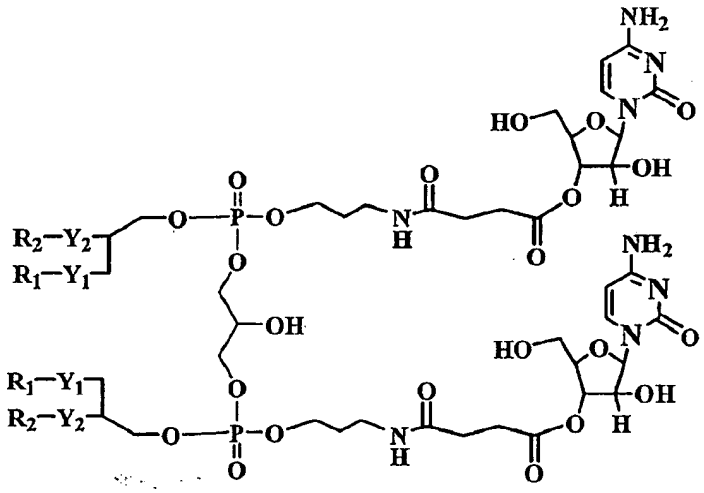
hydroxyl, R_5 is hydrogen, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is –
$CH_2CH_2CH_2NHCOCH_2CH_2C(O)$-group,



XXXIX

and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0068] In a more preferred embodiment, the compound, according to general formula IV, is a gemcitabine-cardiolipin conjugate having structure XXXX, wherein R_3 and R_7 are cytosine, R_4 and R_6 are hydroxyl, R_5 is hydrogen, Y_1, Y_2 are oxo (-O-) or –O-C(O)- groups, the linker is –
$CH_2CH_2CH_2NHCOCH_2CH_2C(O)$-group, and



XXXX

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

[0069] Nucleoside-lipid conjugates can be prepared by any desired method. One preferred method of the present invention is set forth in Figure 2, which depicts a general approach to the synthesis of a gemcitabine-cardiolipin conjugate. In this method, a cardiolipin derivative **6** is synthesized by reacting 1,2-disubsituted glycerol **1** with *N,N,*-diisopropylmethylphosphonamidic chloride **2** in the presence of a base (for example, *N,N,* diisopropylethylamine (DIPEA) and the like) and subsequently with 2-substituted glycerol, (for example, 2-benzylglycerol and the like) in the presence of 1H-tetrazole, followed by oxidation with an mild oxidizing agent such as m-chloroperoxybenzoic acid (mCPBA) or hydrogen peroxide (H_2O_2) (see Ahmad *et al* PCT/US2003/16412). Intermediate **6** is then reacted with succinic anhydride in an inert solvent (for example, 1,2-dichloroethane and the like) in the presence of a base (for example, triethylamine and the like) to provide intermediate **7**. Intermediate **7** is then reacted with 4-*N*-3'-*O*-Bis(*tert*-butoxycarbonyl)gemcitabine **8** (Guo and Gallo, *J. Org. Chem.*, 1999, *64*, 8319-8322) in the presence of dicyclohexylcarbodimide (DCC), *N'N'*-dimethyaminopyridine (DMAP) and an inert solvent (such as dichloromethane and the like). The protecting groups are removed in an acidic medium (such as trifluoroacetic acid (TFA) and the like) in dichloromethane to provide gemcitabine-cardiolipin-conjugate **10**.

[0070] Figure 3 describes another embodiment for synthesizing gemcitabine-cardiolipin conjugates as diammonium salts in which 1,2-disubstituted glycerol **1** is reacted with a phosphoramidite reagent of the general formula **11** (X = phosphate protecting group, preferably a benzyl, 2-cyanoethyl or methyl) in the presence of 1H-tetrazole in dichloromethane and subsequently with 2-substituted glycerol **12** followed by oxidation with mCPBA to give intermediate **14**. The protecting group can be removed by any suitable agent depending the nature of protecting group (for example, tert-butyldimethylsilyl (TBDMS) group can be removed with an acid, benzyl group by hydrogenation) to give intermediate **15**. Intermediate **15**, on reacting with succinic anhydride and later with 4-*N*-3'-*O*-Bis(*tert*-butoxycarbonyl)gemcitabine **8**, gives **17**. The protecting groups (*tert*-butoxy carbonyl, t-BOC) are removed with trifluoroacetic acid in dichloromethane. The phosphate protecting groups can be removed with any suitable reagent depending on the nature of group (for example, a benzyl group can be removed by catalytic hydrogenation, and a methyl group can be removed by heating with sodium iodide in the presence of 2-butanone or acetone). The removal of protecting groups is followed by a treatment with dilute ammonium hydroxide to furnish gemcitabine-cardiolipin conjugate **18** as a diammonium salt.

[0071] Another embodiment for synthesizing gemcitabine-cardiolipin conjugates as diammonium salts is depicted in Figure 4. In this method, an optically pure 1,2-disubstituted glycerol **1** can be phosphorylated using phosphoramidite reagent **19** to yield phosphate triesters which are then reacted with any suitable 2-*O*-protected glycerol, such as, for example, 2-(*tert*-butyldimethylsily)glycerol **20** or 2-levulinoyl-1,3-propane diol, using pyridinium perbromide and phosphonium salt methodology, to get

cardiolipin 22 (see Ahmad *et al* PCT/US2003/027806). Reacting 22 with succinic anhydride followed by coupling with 4-*N*-3'-*O*-Bis(*tert*-butoxycarbonyl)gemcitabine 8 gives 24. Removal of the *tert*-butoxycarbonyl group can be done using an acid catalyst (such as TFA or the like) and the benzyl groups can be removed by catalytic hydrogenation. This is followed by treatment with dilute ammonium hydroxide.

[0072] Another embodiment for synthesizing gemcitabine-cardiolipin conjugates as diammonium salts is depicted in Figure 5. This embodiment involves reaction of cardiolipin intermediate 7 with 4-*N*-5'-*O*-Bis(*tert*-butoxycarbonyl)gemcitabine 26 in the presence of DCC and DMAP in an inert solvent (for example, dichloromethane and the like) followed by deprotection using TFA to give gemcitabine-cardiolipin conjugate 28.

[0073] Another embodiment for synthesizing gemcitabine-cardiolipin conjugates as diammonium salts is depicted in Figure 6. Cardiolipin derivative 16, on reacting with 4-*N*-5'-*O*-Bis(*tert*-butoxycarbonyl)gemcitabine 26, gives 29. The protecting groups (*tert*-butoxycarbonyl) are removed with trifluoroacetic acid in dichloromethane. The protecting groups on the phosphates can be removed with any suitable reagent depending on the nature of group (for example, a benzyl group can be removed by catalytic hydrogenation, and a methyl group can be removed by heating with sodium iodide in the presence of 2-butanone or acetone). This removal is followed by a treatment with dilute ammonium hydroxide to furnish gemcitabine-cardiolipin conjugate 30 as a diammonium salt.

[0074] Another embodiment for synthesizing gemcitabine-cardiolipin conjugates as diammonium salts 33 is depicted in Figure 7. In this method, coupling cardiolipin with 4-*N*-5'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine 26 gives 31. Removal of *tert*-butoxycarbonyl group can be done using an acid catalyst (such as TFA or the like) and the benzyl groups can be removed by catalytic hydrogenation. This is followed by treatment with dilute ammonium hydroxide.

[0075] Another embodiment of the present invention is depicted in Figure 8. In this method, cardiolipin analogue 6 is reacted with *N,N*,-diisopropylmethylphosphonamidic chloride 2 in the presence of a base (for example, DIPEA or the like) in an inert solvent (for example dichloromethane or the like) and then reacted with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine 8 to give fully protected gemcitabine-cardiolipin conjugate 34. Removal of the t-BOC groups with a suitable acid (like TFA or the like) affords gemcitabine-cardiolipin conjugate 35.

[0076] Another embodiment of the present invention is set forth in Figure 9. In this method, cardiolipin analogue 6 is reacted with benzyl tetraisopropylphosphoramidite 36 in the presence of 1H-tetrazole and subsequently with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine followed by oxidation with

a suitable oxidizing agent (for example, mCPBA or the like). Removal of t-BOC groups with TFA and benzyl groups by catalytic hydrogenation, followed by subsequent treatment with dilute ammonium hydroxide, provides conjugate **39** as an ammonium salt.

[0077] Another embodiment of the present invention is outlined in Figure 10, which illustrates the synthesis of a gemicitabine-cardiolipin conjugate as a diammnoium salt. In this method, cardiolipin analogue **22** is reacted with *N,N,*-diisopropylmethylphosphonamidic chloride **2** in the presence of a base (for example, DIPEA or the like) in an inert solvent (for example. dichloromethane or the like) and later with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine **8** in presence of 1H-tetrazole followed by oxidation with a suitable oxidizing agent (for example, mCPBA or the like) to give protected gemcitabine-cardiolipin conjugate **40**. Removal of t-BOC groups with TFA, and removal of the benzyl groups by catalytic hydrogenation followed by treatment with dilute ammonium hydroxide gives conjugate **42** as a diammonium salt.

[0078] Another embodiment of the present invention, represented in Figure 11, leads to the synthesis of a gemcitabine-cardiolipin conjugate as a triammonium salt, wherein cardiolipin intermediate **22** is first reacted with benzyl tetraisopropyl phosphoramidite **36** in the presence of 1H-tetrazole and then subsequently reacted with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine **8** to give protected conjugate **43**. Removal of t-BOC groups with TFA and benzyl groups by catalytic hydrogenation followed by treatment with dilute ammonium hydroxide provides gemcitabine-cardiolipin conjugate **45** as a triammoniun salt.

[0079] Another embodiment of the present invention is represented in Figure 12. In this method, cardiolipin analogue **6** is first reacted with *N,N,*-diisopropylmethylphosphonamidic chloride **2** in the presence of a base (for example, DIPEA or the like) in an inert solvent (for example dichloromethane or the like) and then reacted with 4-*N*-5'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine **26** to give fully protected gemcitabine-cardiolipin conjugate **46**. Removal of the t-BOC groups with a suitable acid (like TFA or the like) affords gemcitabine-cardiolipin conjugate **47**.

[0080] Another embodiment of the present invention is set forth in Figure 13, which depicts an approach for synthesizing a gemcitabine-cardiolipin conjugate as an ammonium salt. In this method, cardiolipin analogue **6** is reacted with benzyl tetraisopropylphosphoramidite **36** in the presence of 1H-tetrazole and subsequently with 4-*N*-5'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine **26** followed by oxidation with suitable oxidizing agent (for example, mCPBA or the like). Removal of t-BOC groups with TFA and benzyl groups by catalytic hydrogenation, followed by subsequent treatment with dilute ammonium hydroxide, provides conjugate **50** as an ammonium salt.

[0081] Another embodiment of the present invention is outlined in Figure 14, which illustrates the synthesis of gemicitabine-cardiolipin conjugate as a diammnoium salt. In this method, cardiolipin analogue **22** is reacted with *N,N*,-diisopropylmethylphosphonamidic chloride **2** in the presence of a base (for example, DIPEA or the like) in an inert solvent (for example. dichloromethane or the like) and later with 4-*N*-5'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine **26** in the presence of 1H-tetrazole followed by oxidation with a suitable oxidizing agent (for example, mCPBA or the like) to give protected gemcitabine-cardiolipin conjugate **51**. Removal of the t-BOC groups with TFA furnishes **52**. The benzyl groups on phosphates can be removed by catalytic hydrogenation. Treatment with dilute ammonium hydroxide gives conjugate **53** as a diammonium salt.

[0082] Another embodiment of the present invention is represented in Figure 15. Cardiolipin intermediate **22** is first reacted with benzyl tetraisopropylphosphoramidite **36** in the presence of 1H-tetrazole and then subsequently reacted with 4-*N*-5'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine **26** to give protected conjugate **54**. Removal of the t-BOC groups with TFA and benzyl groups by catalytic hydrogenation followed by treatment with dilute ammonium hydroxide provides gemcitabine-cardiolipin conjugate **56** as a triammoniun salt.

[0083] Another embodiment of the present invention is represented in Figure 16. In this scheme, optically pure 1,2-disubstituted glycerol **1** is reacted with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite **57**, in the presence of a base (for example, DIPEA or the like) in an inert solvent (for example, dichloromethane or the like), and subsequently reacted with 2-*O*-protected glycerol (for example, 2-*O*-*ter*-butyldimethylsilyl glycerol **20**) in the presence of 1H-tetrazole followed by oxidation with mCPBA which provides cardiolipin derivative **59**. Conversion of cyano to amine functionalities can be achieved by hydrogenation using a suitable catalyst (for example, Pd/C or Raney Ni). The resulting amine on reaction with succinic anhydride furnishes **61**. Intermediate **61** on coupling with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine **8**, followed by deprotection in an acidic condition (for example, TFA or the like), provides gemcitabine-cardiolipin conjugate **63**.

[0084] Another embodiment of the present invention is shown in Figure 17, in which cardiolipin derivative **61** is first reacted with 4-*N*-5'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine **26** and then deprotected with TFA. This reaction results in gemcitabine-cardiolipin conjugate **65**.

[0085] The synthetic methods described herein can be modified in any suitable manner. For example, the protection of gemcitabine is not limited to t-BOC groups but also includes benzyloxycarbonyl (Cbz), trityl benzyloxymethyl, trimethylsilyl, tert-butyldimethylsilyl, benzoyl, acetyl, pivaloyl, levulinoyl and the like. The deprotection can be acheived by a method depending on the protecting group. For example, removal of t-BOC groups is not limited to TFA but also includes HCl and the like. A benzyl

group can be removed by catalytic hydrogenolysis or by treatment with sodium iodide, and a silyl group can be deprotected with fluoride or an acidic medium. In addition, the protecting group of a cardiolipin derivative is not limited to only benzyl and methyl but also includes phosphate, 2-cyanoethyl, ethyl, ter-butyldimethylsilyl, levulinoyl, pivaloyl, methoxymethyl, benzyloxymethyl and the like.

[0086] The described methods can be used to prepare a variety of novel nucleoside-cardiolipin conjugates. For example, the methods can be used to prepare a nucleoside-cardiolipin conjugate in a pure form containing any fatty acid chains. Preferred fatty acids range from carbon chain lengths of about C_1 to C_{34}, preferably between about C_4 and about C_{24}, and include tetranoic acid ($C_{4:0}$), pentanoic acid ($C_{5:0}$), hexanoic acid ($C_{6:0}$), heptanoic acid ($C_{7:0}$), octanoic acid ($C_{8:0}$), nonanoic acid ($C_{9:0}$), decanoic acid ($C_{10:0}$), undecanoic acid ($C_{11:0}$), dodecanoic acid ($C_{12:0}$), tridecanoic acid ($C_{13:0}$), tetradecanoic (myristic) acid ($C_{14:0}$), pentadecanoic acid ($C_{15:0}$), hexadecanoic (palmatic) acid ($C_{16:0}$), heptadecanoic acid ($C_{17:0}$), octadecanoic (stearic) acid ($C_{18:0}$), nonadecanoic acid ($C_{19:0}$), eicosanoic (arachidic) acid ($C_{20:0}$), heneicosanoic acid ($C_{21:0}$), docosanoic (behenic) acid ($C_{22:0}$), tricosanoic acid ($C_{23:0}$), tetracosanoic acid ($C_{24:0}$), 10-undecenoic acid ($C_{11:1}$), 11-dodecenoic acid ($C_{12:1}$), 12-tridecenoic acid ($C_{13:1}$), myristoleic acid ($C_{14:1}$), 10-pentadecenoic acid ($C_{15:1}$), palmitoleic acid ($C_{16:1}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$), eicosenoic acid ($C_{20:1}$), eicosdienoic acid ($C_{20:2}$), eicosatrienoic acid ($C_{20:3}$), arachidonic acid (*cis*-5,8,11,14-eicosatetraenoic acid), and *cis*-5,8,11,14,17-eicosapentaenoic acid, among others. For ether analogs, the alkyl chain will also range from C_1 to C_{34} preferably between about C_4 and about C_{24}. Other fatty acid chains also can be employed as R_1 and/or R_2 substituents. Examples of such include saturated fatty acids such as ethanoic (or acetic) acid, propanoic (or propionic) acid, butanoic (or butyric) acid, hexacosanoic (or cerotic) acid, octacosanoic (or montanic) acid, triacontanoic (or melissic) acid, dotriacontanoic (or lacceroic) acid, tetratriacontanoic (or gheddic) acid, pentatriacontanoic (or ceroplastic) acid, and the like; monoethenoic unsaturated fatty acids such as *trans*-2-butenoic (or crotonic) acid, *cis*-2-butenoic (or isocrotonoic) acid, 2-hexenoic (or isohydrosorbic) acid, 4-decanoic (or obtusilic) acid, 9-decanoic (or caproleic) acid, 4-dodecenoic (or linderic) acid, 5-dodecenoic (or denticetic) acid, 9-dodecenoic (or lauroleic) acid, 4-tetradecenoic (or tsuzuic) acid, 5-tetradecenoic (or physeteric) acid, 6-octadecenoic (or petroselenic) acid, *trans*-9-octadecenoic (or elaidic) acid, *trans*-11-octadecenoic (or vaccinic) acid, 9-eicosenoic (or gadoleic) acid, 11-eicosenoic (or gondoic) acid, 11-docosenoic (or cetoleic) acid, 13-decosenoic (or erucic) acid, 15-tetracosenoic (or nervonic) acid, 17-hexacosenoic (or ximenic) acid, 21-triacontenoic (or lumequeic) acid, and the like; dienoic unsaturated fatty acids such as 2,4-pentadienoic (or β–vinylacrylic) acid, 2,4-hexadienoic (or sorbic) acid, 2,4-decadienoic (or stillingic) acid, 2,4-dodecadienoic acid, 9,12-hexadecadienoic acid, *cis*-9, *cis*-12-octadecadienoic (or α–linoleic) acid,

trans-9, *trans*-12-octadecadienoic (or linlolelaidic) acid, *trans*-10,*trans*-12-octadecadienoic acid, 11,14-eicosadienoic acid, 13,16-docosadienoic acid, 17,20-hexacosadienoic acid and the like; trienoic unsaturated fatty acids such as 6,10,14-hexadecatrienoic (or hiragonic) acid, 7,10,13-hexadecatrienoic acid, *cis*-6, *cis*-9-*cis*-12-octadecatrienoic (or γ–linoleic) acid, *trans*-8, *trans*-10- *trans*-12-octadecatrienoic (or β–calendic) acid, *cis*-8, *trans*-10- *cis*-12-octadecatrienoic acid, *cis*-9, *cis*-12- *cis*-15-octadecatrienoic (or α–linolenic) acid, *trans*-9, *trans*-12- *trans*-15-octadecatrienoic (or α–linolenelaidic) acid, *cis*-9, *trans*-11- *trans*-13-octadecatrienoic (or α–eleostearic) acid, *trans*-9, *trans*-11- *trans*-13-octadecatrienoic (or β–eleostearic) acid, *cis*-9, *trans*-11- *cis*-13-octadecatrienoic (or punicic) acid, 5,8,11-eicosatrienoic acid, 8,11,14-eicosatrienoic acid and the like; tetraenoic unsaturated fatty acids such as 4,8,11,14-hexadecatetraenoic acid, 6,9,12,15- hexadecatetraenoic acid, 4,8,12,15-octadecatetraenoic (or moroctic) acid, 6,9,12,15-octadecatetraenoic acid, 9,11,13,15- octadecatetraenoic (or α –or β-parinaric) acid, 9,12,15,18-octadecatetraenoic acid, 4,8,12,16-eicosatetraenoic acid, 6,10,14,18-eicosatetraenoic acid, 4,7,10,13-docasatetraenoic acid, 7,10,13,16-docosatetraenoic acid, 8,12,16,19-docosatetraenoic acid and the like; penta- and hexa-enoic unsaturated fatty acids such as 4,8,12,15,18-eicosapentaenoic (or timnodonic) acid, 4,7,10,13,16-docosapentaenoic acid, 4,8,12,15,19-docosapentaenoic (or clupanodonic) acid, 7,10,13,16,19-docosapentaenoic, 4,7,10, 13,16,19-docosahexaenoic acid, 4,8,12,15,18,21-tetracosahexaenoic (or nisinic) acid and the like; branched-chain fatty acids such as 3-methylbutanoic (or isovaleric) acid, 8-methyldodecanoic acid, 10-methylundecanoic (or isolauric) acid, 11-methyldodecanoic (or isoundecylic) acid, 12-methyltridecanoic (or isomyristic) acid, 13-methyltetradecanoic (or isopentadecylic) acid, 14-methylpentadecanoic (or isopalmitic) acid, 15-methylhexadecanoic, 10-methylheptadecanoic acid, 16-methylheptadecanoic (or isostearic) acid, 18-methylnonadecanoic (or isoarachidic) acid, 20-methylheneicosanoic (or isobehenic) acid, 22-methyltricosanoic (or isolignoceric) acid, 24-methylpentacosanoic (or isocerotic) acid, 26-methylheptacosanoic (or isomonatonic) acid, 2,4,6-trimethyloctacosanoic (or mycoceranic or mycoserosic) acid, 2-methyl-*cis*-2-butenoic(angelic)acid, 2-methyl-*trans*-2-butenoic (or tiglic) acid, 4-methyl-3-pentenoic (or pyroterebic) acid and the like. For ether analogues, the alkyl chain also will range from carbon chain lengths of C_1 to C_{34}.

[0087] The described methods can be used to prepare a variety of novel nucleoside-cardiolipin conjugates in a pure form containing any alkyl or acyl chain ranging from C_1-C_{34} chain lengths. Preferred alkyl chains range from carbon chain lengths of about $C_1$to C_{24}. The term "alkyl" encompasses saturated or unsaturated straight-chain and branched chain hydrocarbon moieties. The term "substituted alkyl" or

substituted "alkoxy" and the like includes alkyl or alkoxy groups further bearing one or more substitutent selected from hydroxyl, alkoxy (of lower alkyl group), halogen, cyano, and the like.

[0088] Nucleoside-lipid conjugates which are compatible with the present invention include nucleosides which act on the peripheral nerves, adrenergic receptors, cholinergic receptors, the skeletal muscles, the cardiovascular system, smooth muscles, the blood circulatory system, synaptic sites, neuroeffector junctional sites, endocrine and hormone systems, the immunological system, the reproductive system, the skeletal system, the alimentary and excretory systems, the histamine system and the central nervous system. Active nucleosides of nucleoside-lipid conjugates of the present invention can be analgesics, anesthetics, anti-arrythmic, antiallergic, antifungal, anticancer (e.g. gemcitabine, cytarabine) antihypertensive, anticoagulants; antidepressants; antidiabetic, anti-epilepsy, anti-inflammatory, antiulcer, anti-protozoal, antivirals, or nucleoside-lipid conjugates for treating demyelinating diseases including multiple sclerosis; ophthalmic agents; vaccines (e.g., against influenza virus, pneumonia, hepatitis A, hepatitis B, hepatitis C, cholera toxin B-subunit, typhoid, plasmodium falciparum, diptheria, tetanus, herpes simplex virus, tuberculosis, HIV, bordetela pertusis, measles, mumps, rubella, bacterial toxoids, vaccinea virus, adenovirus, SARS virus, canary virus, bacillus calmette Guerin, klebsiella pneumonia vaccine, etc.); histamine receptor antagonists, hypnotics, kidney protective agents, lipid regulating agents, muscle relaxants, neuroleptics, neurotropic agents, opioid agonists and antagonists, parasympathomimetics, protease inhibitors, prostglandins, sedatives, sex hormones (e.g., androgens, estrogens, etc.), stimulants, sympathomimetics, vasodilators and xanthins and synthetic analogs of these species.

[0089] The new nucleoside-lipid conjugates of this invention are potentially useful for the treatment of cancer. The cancer can be any type of cancer in a mammal. Examples include, but are not limited to, cancers of the head, neck, brain, blood (e.g. leukemia, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, lymphoma, myeloma), breast, lung, pancreas, bone, spleen, bladder, prostate, testes, colon, kidney ovary and skin (e.g. Kaposi's sarcoma). In addition, the nucleoside-lipid conjugates of the present invention are useful in reducing the tendency of cancers cells to develop a resistance to other therapeutic agents such as anti-cancer agents, chemotherapy and radiation.. Thus, other therapeutic agents can be advantageously employed with the present invention in the formation of an active combination or by separate administration.

[0090] The new nucleoside-lipid conjugates of this invention can also be potentially useful in the treatment of viral diseases such as HIV, herpes simplex viruses (HSV1 and HSV2), human herpes virus 6, human herpes virus 7, human herpes virus 8, orthopoxviruses (e.g. variola major and minor, small pox),

ebola virus, influenza virus, tuberculosis, hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E, hepatitis G, parainfluenza virus, respiratory syncytial virus, cholera, pneumonia, SARS virus, canary virus, West Nile virus (WNV), respiratory syncytial virus (RSV), dengue virus, vericella zoster virus, corona viruses, vaccinia virus, cytomegalovirus (CMV), human rhinovirus (HRV), papilloma virus (PV) and Epstein Barr virus.

[0091] It should be noted that, where the inventive nucleoside-lipid conjugates are employed to treat or alleviate diseases (e.g., cancer, viral) in human or animal patients, they need not result in a complete cure or remission of the disease to be shown to be successfully employed. In addition, as used herein, "alleviating a disease" means reducing the severity of a symptom of the disease. As used herein, "treating a disease" means reducing the frequency with which a symptom of the disease is experienced by a mammal. Thus, for example, the disease may be treated by using the inventive nucleoside-lipid conjugate, the progress of the disease is slowed or retarded in the patient. Alternatively, the disease may be considered to be treated if, for adjunctive uses, the inventive nucleoside-lipid conjugate renders the disease more amenable to other treatments or demonstrates additive, but not necessarily synergistic, therapeutic potential as compared to monotherapy using other treatment regimens. In some embodiments, the use of the nucleoside-lipid conjugates, in accordance with the present invention, can lead to remission of cancer or other diseases.

[0092] The new nucleoside-lipid conjugates of this invention are potentially useful in the treatment or alleviation of bone disorders. These conjugates can be used in methods for inhibiting bone resorption, methods for increasing bone formation by preventing osteoblast and osteocyte apoptosis and methods for increasing bone mass and strength. Indications for their use include the treatment or alleviation of osteoporosis, Paget's disease, metastatic bone cancers, hyperparathyroidism, rheumatoid arthritis, algodistrophy, sterno-costo-clavicular hyperostosis, Gaucher's disease, Engleman's disease and certain non-skeletal disorders.

[0093] The nucleoside-lipid conjugates of the present invention can be administered intravenously, subcutaneously, locally, orally, parenterally, intraperitoneally, and/or rectally, nasally, vaginally, lingually or by direct injection into tumors or sites in need of treatment by such as are known or developed. The present pharmaceutical preparations can contain the nucleoside-lipid conjugate alone or can contain further substances of pharmaceutical importance. They can further comprise a pharmaceutically acceptable carrier.

[0094] Tablets, dragees, capsules, pills, granules, suppositories, solutions suspensions and emulsions, pastes, ointments, gels, creams, lotions, powders and sprays can be suitable pharmaceutical preparations. Suppositories can contain, in addition to the nucleoside-lipid conjugate, suitable water soluble or water-

insoluble excipients. Suitable excipients are those in which the inventive nucleoside-lipid conjugate is sufficiently stable to allow for therapeutic use, for example polyethylene glycols, certain fats, and esters or mixture of substances. Ointments, pastes, creams and gels can also contain suitable expcipients in which the lipid-nucleoside conjugate is stable.

[0095] Another use for the inventive nucleoside-lipid conjugate is in the preparation of liposomes and other lipid-containing formulations. In addition to the nucleoside-lipid conjugate, the invention includes a composition, wherein the liposome further comprises phospholipids and/or lysophospholipids selected from a group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, cholesterol, α-tocopherol, natural or synthetic cardiolipin and analogues thereof, synthetic cationic cardiolipin analogues and derivatives thereof, phosphatidylserine, phosphatidylinositol, sphingomyelin, lysophosphotidylglycerol, lysophosphatidic acid, lysophosphotidylcholine, lysophosphatidylserine and PEG modified lipids.

[0096] Liposomes, according to the present invention, can be multilamellar vesicles, unilamellar vesicles, or a mixture thereof. Moreover, the liposomes can be of varying size or can be substantially uniform in size. For example, the liposomes can have a size range of about 1 mm or less, and more preferably are in the micron or sub-micron range. For example, the liposomes can have a diameter of about 5μm or less, such as about 1μm or less, or even about 0.5μm or less, such as about 0.2μm or less or even about 0.1μm or less.

[0097] The inventive nucleoside-lipid conjugates can be employed in the form of a combination with other therapeutic agents. The therapeutic agent can become complexed with a portion of the lipid (such as the inventive nucleoside-lipid conjugate) or the therapeutic agent can become entrapped within the liposomes. Alternatively, the therapeutic agent can be administered separately. In addition, a second therapeutic agent can be administered adjunctively, prior to, concurrently with, or after the first therapeutic agent. Preferred agents include antineoplastic, antifungal, antibiotic and other therapeutic agents, particularly cisplatin, antisense oligonucleotides, siRNA, oxaliplatin, paclitaxel, vinorelbine and epirubicin.

[0098] In addition to synthetic nucleoside-lipid conjugates, liposomes can include stabilizers, absorption enhancers, antioxidants, phospholipids, biodegradable polymers, and medicinally active agents among other ingredients. The amount of nucleoside-lipid conjugates in liposomes can be controlled by varying the composition of lipids and/or other components in it.

[0099] In some embodiments, it is preferable for liposomes to also include targeting agents such as a carbohydrate, a protein, ligands that bind to a specific substrate (such as antibodies or fragments thereof),

or ligands that recognize cellular receptors. The inclusion of such agents (such as a carbohydrate, or one or more proteins selected from a group of proteins consisting of antibodies, antibody fragments, peptide, peptide hormones, receptor ligands, such as an antibody to a cellular receptor, and mixtures thereof) can facilitate targeting the liposome to a predetermined tissue or cell type.

[00100] Liposomes, according to the present invention, can be prepared by any suitable technique. Lipophilic liposome-forming ingredients, such as phosphatidylcholine, a nucleoside-lipid conjugate prepared by the methods described above, cholesterol and α-tocopherol can be dissolved or dispersed in a suitable solvent or combination of solvents and dried. Suitable solvents include any non-polar or slightly polar solvent, such as *t*-butanol, ethanol, methanol, chloroform, or acetone that can be evaporated without leaving a pharmaceutically unacceptable residue. Drying can be by any suitable means such as by lyophilization. Hydrophilic ingredients can be dissolved in polar solvents, including water.

[00101] Liposomes can be formed by mixing the dried lipophilic ingredients with the hydrophilic mixture. Mixing the polar solution with the dried lipid film can be by any means that strongly homogenizes the mixture. The homogenization can be effected by vortexing, magnetic stirring and/or sonicating. Once formed, the liposomes can be filtered through suitable filters to control their size distribution. Suitable filters include these that can be used to obtain the desired size range of liposomes from a filtrate. For examples, the liposomes can be formed and thereafter filtered through a 5 micron filter to obtain liposomes of about 5 microns or less. Alternatively, 1 μm, 500 nm, 100 nm or other suitable filters can be used to obtain liposomes of a desired size. The present inventive liposomes can also be filtered through microbial retentative filters to obtain a sterile pharmaceutical product.

[00102] The invention also includes a kit for administering the composition of the present invention to a mammal for the treatment or alleviation of a disease. The disease can be any one or more of the diseases described herein. The kit comprises the composition of the invention and an instructional manual which describes the administration of the composition to a mammal by any of the routes of administration described herein. In another embodiment, this kit comprises a solvent, preferably sterile solvent, suitable for dissolving or suspending the composition of the invention prior to administering the composition to the mammal.

[00103] All references, including publications, patent applications, and patents cited herein, including those in the preceding list and otherwise cited in this specification, are hereby incorporated by reference to the same extent as if each reference is were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[00104] The use the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specifications should be construed as indicating any non-claimed element as essential to the practice of the invention.

[00105] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments can become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the inventions to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[00106] The following specific examples further illustrate the invention but, of course, should not be construed as limiting its scope in any way.

Example 1

Synthesis of a Gemcitabine Cardiolipin Conjugate

[00107] First, commercially available (*R*)-(+)-3-benzyloxy-1,2-propanediol was reacted with 1-bromohexane in the presence of sodium hydride (60% in oil dispersion) in dimethyl formamide (DMF). This reaction was followed by debenzylation *via* catalytic hydrogenolysis (H_2/Pd-C) which provided 1,2-di-*O*-hexyl-*sn*-glycerol in a 89% yield. The next step involved reacting 1,2-di-*O*-hexyl-*sn*-glycerol with a bifunctional phosphitylating reagent *N,N*-diisopropylmethylphosphonamidic chloride in the presence of *N,N*-diisopropylethylamine (DIPEA) in dichloromethane at room temperature to give 1,2-di-*O*-hexyl-*sn*-glycero-*N,N*-diisopropyl methylphosphoramidite. 1,2-di-*O*-hexyl-*sn*-glycero-*N,N*-diisopropyl methylphosphoramidite was then reacted with 2-benzyloxy-1,3-propanediol in the presence of 1*H*-tetrazole

to provide a phosphite triester. In situ oxidation of the phosphite trimester, with *m*-chloroperbenzoic acid (*m*CPBA) at -40 °C, afforded 2-*O*-benzyl-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether in the form of a colorless oil. A yield of 79% was achieved after purification of the 2-*O*-benzyl-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether on a silica gel column (hexane-ethyl acetate, 8:2-6:4). Purification was followed by hydrogenolysis by reacting 2-*O*-benzyl-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether with H_2/Pd-C at 50 psi for 2 hours. This reaction furnished a 96% yield of 1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether in the form of a colorless oil. The central hydroxyl functionality of 1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether (cardiolipin analogue) was then reacted with succinic anhydride in the presence of triethylamine and 4-dimethylamino pyridine (DMAP) in 1,2-dichloroethane to provide a 79% yield of 1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)-2-succinylglycerol dimethyl ether. 1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)-2-succinylglycerol dimethyl ether was then reacted with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine in the presence of *N,N*-dicyclohexyl carbodimide (DCC) and DMAP in dichloromethane at room temperature for 8 hours. This reaction was followed by purification on a silica gel column (hexane-ethyl acetate, 7:3-4:6) which afforded a 84% yield of 4-*N*-3'-*O*-di(tert-butoxycarbonyl)-5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero)-3-phosphoryl glycerol dimethyl ether] gemcitabine. The protecting groups of 4-*N*-3'-*O*-di(tert-butoxycarbonyl)-5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero)-3-phosphoryl glycerol dimethyl ether] gemcitabine were removed using trifluororacetic acid (TFA) in dichloromethane at room temperature. The reaction solution was neutralized with 5% aqueous sodium bicarbonate at 0 °C, extracted with dichloromethane and then concentrated. Purification of the crude compound on a silica gel column (chloroform-methanol, 98:2-96:4) afforded an 80% yield of pure 5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero)-3-phosphorylglycerol dimethyl ether]gemcitabine in the form of a colorless viscous oil. This final product was characterized by ^{1}H NMR, ^{13}C NMR, IR and Mass Spectroscopy. The purity was checked by HPLC and elemental analysis.

Example 2

[00108] Alkylation of commercially available (R)-(+)-2,2-dimethyl-1,3-dioxolane-methanol with 1-bromohexane in the presence of sodium hydride (60% in an oil dispersion) in tetrahydrofuran (THF) followed by deprotection of the isopropylidene group with 2N HCl in methanol provided 1-*O*-hexyl-glycerol. Tritylation of 1-*O*-hexyl-glycerol,using trityl chloride in the presence of triethylamine and DMAP in dichloromethane, gave 1-O-hexyl-3-*O*-trityl glycerol. Methylation of the trityl derivative with iodomethane, in the presence of sodium hydride in THF, followed by detritylation with p-toluenesulfonic acid monohydrate, in THF, provided 1-*O*-hexyl-2-*O*-methylglycerol with a 63% yield. The next step involved the reaction of 1-*O*-hexyl-2-*O*-methyl-*sn*-glycerol with *N,N*-diisopropylmethylphosphonamidic

chloride in the presence of *N,N*-diisopropylethylamine (DIPEA) in dichloromethane at room temperature. This reaction resulted in 1-*O*-hexyl-2-*O*-methyl-*sn*-glycero-*N,N*-diisopropyl methylphosphoramidite which was then reacted with 2-benzyloxy-1,3-propanediol, in the presence of 1*H*-tetrazole, to provide a phosphite triester. In situ oxidation of the phosphite trimester, with *m*-chloroperbenzoic acid (*m*CPBA) at -40 °C, afforded 2-*O*-benzyl-1,3-bis-(1-*O*-hexyl-2-*O*-methyl--*sn*-glycero-3-phosphoryl)glycerol dimethyl ether. Purification of this product on a silica gel column (hexane-ethyl acetate, 8:2-4:6) resulted in 2-*O*-benzyl-1,3-bis-(1-*O*-hexyl-2-*O*-methyl--*sn*-glycero-3-phosphoryl)glycerol dimethyl ether as a colorless oil in 87% yield. Hydrogenolysis of 2-*O*-benzyl-1,3-bis-(1-*O*-hexyl-2-*O*-methyl--*sn*-glycero-3-phosphoryl)glycerol dimethyl ether, with H_2/Pd-C at 50 psi for 2 hours, resulted in 1,3-bis-(1-*O*-hexyl-2-*O*-methyl--*sn*-glycero-3-phosphoryl)glycerol dimethyl ether as a colorless oil in 96% yield. The central hydroxyl functionality of 1,3-bis-(1-*O*-hexyl-2-*O*-methyl--*sn*-glycero-3-phosphoryl)glycerol dimethyl ether (cardiolipin analogue) was reacted with succinic anhydride in the presence of triethylamine and 4-dimethylamino pyridine (DMAP) in 1,2-dichloroethane which provided 1,3-bis-(1-*O*-hexyl-2-*O*-methyl--*sn*-glycero-3-phosphoryl)-2-succinyl glycerol dimethyl ether in a 68% yield. 1,3-bis-(1-*O*-hexyl-2-*O*-methyl--*sn*-glycero-3-phosphoryl)-2-succinyl glycerol dimethyl ether was then coupled with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine in the presence of *N,N*-dicyclohexyl carbodimide (DCC) and DMAP in dichloromethane at room temperature for 8 hours. This was followed by purification on a silica gel column (dichloromethane-acetone, 8:2-6:4) which afforded 4-*N*-3'-*O*-di(tert-butoxycarbonyl)-5'-*O*-succinyl-[2-*O*-1,3-bis-(1-*O*-hexyl-2-*O*-methyl -*sn*-glycero)-3-phosphoryl glycerol dimethyl ether] gemcitabine in a 60% yield. The protecting groups were removed using trifluororacetic acid (TFA) in dichloromethane at room temperature. The reaction solution was neutralized with 5% aqueous sodium bicarbonate at 0 °C, extracted with dichloromethane and concentrated. Purification of the crude compound on a silica gel column (chloroform-methanol, 98:2-9:1) afforded pure 5'-*O*-succinyl-[2-*O*-1,3-bis-(1-*O*-hexyl-2-*O*-methyl -*sn*-glycero)-3-phosphorylglycerol dimethyl ether]gemcitabine as a viscous oil in a 70% yield. The product was characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS. The purity was checked by HPLC.

Example 3

[00109] 1,2-di-*O*-hexyl-*sn*-glycerol was treated with dibenzyl diisopropylphosphoramidite and tetrazole in anhydrous dichloromethane and then reacted with 2-*O*-*tert*-butyldimethylsilyl-1,3-propanediol and pyridinium tribromide in the presence of triethyl amine and pyridine in anhydrous dichloromethane. This reaction was followed by purification on a silica gel column (hexane: ethyl acetate , 9:1 to 7:3) to give 2-*O*-tetrabutyldimethyl silyl-1,3-bis [(1, 2-di-*O*-hexyl-*sn*-glycero-3)-phosphoryl] glycerol dibenzyl ether in a 60% yield. The silyl protecting group was removed using 7:3 mixture of ethanol: HCL to give 1, 3-bis [(1, 2-di-*O*-hexyl-*sn*-glycero-3)-phosphoryl] glycerol dibenzyl ether. The central hydroxyl functionality of 1, 3-bis [(1, 2-di-*O*-hexyl-*sn*-glycero-3)-phosphoryl] glycerol dibenzyl ether (cardiolipin analogue) was reacted with succinic anhydride in the presence of triethylamine and 4-dimethylamino pyridine (DMAP) in

1,2-dichloroethane to provide 1,3-bis-(1, 2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)-2-succinyl glycerol dibenzyl ether with a 78% yield. 1,3-bis-(1, 2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)-2-succinyl glycerol dibenzyl ether was coupled with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine in the presence of *N,N*-dicyclohexyl carbodimide (DCC) and DMAP in dichloromethane at room temperature for 8 hours. This was followed by purification on a silica gel column (dichloromethane-methanol, 9:5-0:5) which afforded 4-*N*-3'-*O*-di(tert-butoxycarbonyl)-5'-*O*-succinyl-[2-*O*-1,3-bis-(1-*O*-hexyl-*sn*-glycero)-3-phosphoryl glycerol dimethyl ether] gemcitabine in a 71% yield. The t-BOC protecting groups were removed using trifluororacetic acid (TFA) in dichloromethane at room temperature. The reaction solution was neutralized with 5% aqueous sodium bicarbonate at 0 °C, extracted with dichloromethane and concentrated. Purification of the crude compound on a silica gel column (chloroform-methanol, 98:2-9:1) afforded pure 5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero)-3-phosphorylglycerol dibenzyl ether]gemcitabine as a colorless viscous oil with a 64% yield. The benzyl groups were removed by hydrogenation using Pd/C in ethanol at 30 psi for 3 hrs. The catalyst was removed by filtration on celite. A few drops of ammonium hydroxide were added to the filtrate and concentrated. The crude product was crystallized with methylene chloride and acetone to provide 5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero)-3-phosphorylglycerol diammonium salt]gemcitabine. The product was then characterized by ^{1}H NMR.

Example 4

[00110] 1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)-2-succinylglycerol dimethyl ether was coupled with 4-*N*-5'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine in the presence of *N,N*-dicyclohexyl carbodimide (DCC) and DMAP in dichloromethane at room temperature for 8 hours. This reaction was followed by purification on a silica gel column (hexane-ethyl acetate, 7:3-4:6) which afforded 4-*N*-5'-*O*-di(tert-butoxycarbonyl)-3'-*O*-succinyl-[2-*O*-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero)-3-phosphoryl glycerol dimethyl ether] gemcitabine in a 84% yield. The protecting groups were removed using trifluororacetic acid (TFA) in dichloromethane at room temperature. The reaction solution was then neutralized with 5% aqueous sodium bicarbonate at 0 °C, extracted with dichloromethane and concentrated. Purification of the crude compound on a silica gel column (chloroform-methanol, 98:2-96:4) afforded pure 3'-*O*-succinyl-[2-*O*-1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero)-3-phosphorylglycerol dimethyl ether]gemcitabine as a viscous oil with a 80% yield. The product was characterized by ^{1}H NMR, ^{13}C NMR, IR and Mass Spectroscopy. The purity was checked by HPLC and elemental analysis.

Example 5

[00111] 1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether was reacted with *N,N*-diisopropylmethylphosphonamidic chloride in the presence of *N,N*-diisopropylethylamine (DIPEA) in dichloromethane at room temperature. The resulting intermediate was subsequently reacted with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine, in the presence of 1*H*-tetrazole, to provide a phosphite triester. *In*

situ oxidation of the phosphite trimester, with *m*-chloroperbenzoic acid (*m*CPBA) at -40 °C, afforded 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)-5'*O*-methyl phosphoryl-[1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether]gemcitabine which was then purified on a silica gel column (methylene chloride:methanol 9.8:0.2). This purification resulted in 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)-5'*O*-methyl phosphoryl-[1,3-bis-(1,2-di-*O*-hexyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether]gemcitabine as a colorless oil with a 60% yield. The protecting groups were removed using trifluororacetic acid (TFA) in dichloromethane at room temperature. The reaction solution was neutralized with 5% aqueous sodium bicarbonate at 0 °C, extracted with dichloromethane and concentrated. Purification of the crude compound on a silica gel column (chloroform-methanol, 98:2-9.5:0.5) afforded pure 5'-*O*-methyl phosphoryl-[2-*O*-1,3-bis-(1-*O*-hexyl-2-*O*-methyl -*sn*-glycero)-3-phosphorylglycerol dimethyl ether]gemcitabine as a viscous oil in a 51% yield. The product was characterized by ^{1}H NMR, and HRMS. The purity was checked by HPLC.

Example 6

[00112] 1,2-*O*-dimyristyl-*sn*-glycerol was reacted with *N,N*-diisopropylmethylphosphonamidic chloride in the presence of *N,N*-diisopropylethylamine (DIPEA) in dichloromethane at room temperature to give 1,2-*O*-dimyristyl-*sn*-glycero-*N,N*-diisopropyl methylphosphoramidite which was subsequently reacted with 2-benzyloxy-1,3-propanediol, in the presence of 1*H*-tetrazole, to provide a phosphite triester. *In situ* oxidation of the phosphite trimester, with *m*-chloroperbenzoic acid (*m*CPBA) at -40 °C, afforded 2-*O*-benzyl-1,3-bis-(1,2- *O*-dimyristyl -*sn*-glycero-3-phosphoryl)glycerol dimethyl ether. 1,3-bis-(1,2- *O*-dimyristyl -*sn*-glycero-3-phosphoryl)glycerol dimethyl ether was then purified on a silica gel column (hexane-ethyl acetate, 1:0-1:1), resulting in a colorless oil with a 90% yield. Hydrogenolysis of 2-*O*-benzyl-1,3-bis-(1,2- *O*-dimyristyl -*sn*-glycero-3-phosphoryl)glycerol dimethyl ether, with H_2/Pd-C at 50 psi for 5 hours, furnished 1,3-bis-(1,2- *O*-dimyristyl -*sn*-glycero-3-phosphoryl)glycerol dimethyl ether as a colorless oil in a 98% yield. The central hydroxyl functionality of 1,3-bis-(1,2-*O*-dimyristyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether (cardiolipin analogue) was reacted with succinic anhydride in the presence of triethylamine and 4-dimethylamino pyridine (DMAP) in 1,2-dichloroethane to provide 1,3-bis-(1,2- *O*-dimyristyl -*sn*-glycero-3-phosphoryl)-2-succinylglycerol dimethyl ether in a 88% yield. 1,3-bis-(1,2-*O*-dimyristyl-*sn*-glycero-3-phosphoryl)-2-succinylglycerol dimethyl ether was coupled with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine in the presence of *N,N*-dicyclohexyl carbodimide (DCC) and DMAP in dichloromethane at room temperature for 8 hours. This reaction was followed by purification on a silica gel column (hexane-ethyl acetate, 7:3-1:1) which afforded 4-*N*-3'-*O*-di(tert-butoxycarbonyl)-5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2- *O*-dimyristyl -*sn*-glycero)-3-phosphoryl glycerol dimethyl ether] gemcitabine with a 73% yield. The protecting groups were removed using trifluororacetic acid (TFA) in dichloromethane at room temperature. The reaction solution was neutralized with 5% aqueous sodium bicarbonate at 0 °C, extracted with dichloromethane and concentrated. Purification of the crude compound on a silica gel

column (chloroform-methanol, 98:2-96:4) afforded pure 5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2- *O*-dimyristyl -*sn*-glycero)-3-phosphorylglycerol dimethyl ether]gemcitabine as a white crystalline solid with a 69% yield. The product was characterized by ^{1}H NMR. The purity was checked by HPLC and elemental analysis.

Example 7

[00113] Alkylation of commercially available (R)-(-)-2,2-dimethyl-1,3-dioxolane-methanol with 1-bromotetradecane in the presence of sodium hydride (60% in oil dispersion) in *N,N*-dimethylformamide (DMF) followed by deprotection of isopropylidene group, with 2N HCl in methanol, provided 1-*O*-tetradecyl-glycerol. Tritylation of 1-*O*-tetradecyl-glycerol was accomplished by using trityl chloride in the presence of triethylamine and DMAP in dichloromethane. This resulted in 1-*O*-tetradecyl-3-*O*-trityl glycerol. The trityl derivative was methylated with iodomethane, in the presence of sodium hydride in THF. This reaction was followed by detritylation with p-toluenesulfonic acid monohydrate in THF which provided 1-*O*-tetradecyl-2-*O*-methylglycerol with a 65% yield. The next step involved the reaction of 1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycerol with *N,N*-diisopropylmethylphosphonamidic chloride, in the presence of *N,N*-diisopropylethylamine (DIPEA) in dichloromethane at room temperature, to give 1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero-*N,N*-diisopropyl methylphosphoramidite which was subsequently reacted with 2-benzyloxy-1,3-propanediol in the presence of 1*H*-tetrazole to provide a phosphite triester. *In situ* oxidation of the phosphite trimester, with *m*-chloroperbenzoic acid (*m*CPBA) at -40 °C, afforded 2-*O*-benzyl-1,3-bis-(1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether which was then purified on a silica gel column (methylene chloride-ethyl acetate, 8:2-6:4). This resulted in 2-*O*-benzyl-1,3-bis-(1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether as a colorless oil with a 68% yield. Hydrogenolysis of 2-*O*-benzyl-1,3-bis-(1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether, with H_2/Pd-C at 50 psi for 2 hours, furnished 1,3-bis-(1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether as a colorless oil with a 96% yield. The central hydroxyl functionality of 1,3-bis-(1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ether (cardiolipin analogue) was reacted with succinic anhydride in the presence of triethylamine and 4-dimethylamino pyridine (DMAP) in 1,2-dichloroethane to provide 1,3-bis-(1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero-3-phosphoryl)-2-succinyl glycerol dimethyl ether with a 79% yield. 1,3-bis-(1-*O*- tetradecyl -2-*O*-methyl--*sn*-glycero-3-phosphoryl)-2-succinyl glycerol dimethyl ether was coupled with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine in the presence of *N,N*-dicyclohexyl carbodimide (DCC) and DMAP in dichloromethane at room temperature for 8 hours. This reaction was followed by purification on a silica gel column (hexane-acetone, 8.5:1.5-7:3) which afforded 4-*N*-3'-*O*-di(tert-butoxycarbonyl)-5'-*O*-succinyl-[2-*O*-1,3-bis-(1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero)-3-phosphoryl glycerol dimethyl ether] gemcitabine with a 60% yield. The protecting groups were removed using trifluororacetic acid (TFA) in dichloromethane at room temperature. The reaction solution was neutralized with 5% aqueous sodium bicarbonate at 0 °C, extracted with dichloromethane and concentrated. Purification of the crude compound on a silica gel

column (chloroform-methanol, 98:2-9.5:0.5) afforded pure 5'-*O*-succinyl-[2-*O*-1,3-bis-(1-*O*-tetradecyl-2-*O*-methyl-*sn*-glycero)-3-phosphorylglycerol dimethyl ether]gemcitabine as a viscous oil with a 70% yield. The product was characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS. The purity was checked by HPLC.

Example 8

[00114] 1,2-*O*-dimyristoyl-*sn*-glycerol was reacted with *N,N*-diisopropylmethylphosphonamidic chloride in the presence of *N,N*-diisopropylethylamine (DIPEA) in dichloromethane at room temperature to give 1,2- *O*-dimyristoyl -*sn*-glycero-*N,N*-diisopropyl methylphosphoramidite which was subsequently reacted with 2-benzyloxy-1,3-propanediol, in the presence of 1*H*-tetrazole, to provide a phosphite triester. *In situ* oxidation of the phosphite trimester, with *m*-chloroperbenzoic acid (*m*CPBA) at -40 °C, afforded 2-*O*-benzyl-1,3-bis-(1,2-*O*-dimyristoyl -*sn*-glycero-3-phosphoryl)glycerol dimethyl ester which was then purified on a silica gel column (hexane-ethyl acetate, 2:1-1:1) resulting in 2-*O*-benzyl-1,3-bis-(1,2-*O*-dimyristoyl -*sn*-glycero-3-phosphoryl)glycerol dimethyl ester as a colorless oil with a 90% yield. Hydrogenolysis of 2-*O*-benzyl-1,3-bis-(1,2-*O*-dimyristoyl -*sn*-glycero-3-phosphoryl)glycerol dimethyl ester, with H_2/Pd-C in ethanol at 40 psi for 3 hours, furnished 1,3-bis-(1,2-*O*-dimyristoyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ester as a colorless oil with a 96% yield. The central hydroxyl functionality of 1,3-bis-(1,2-*O*-dimyristoyl-*sn*-glycero-3-phosphoryl)glycerol dimethyl ester (cardiolipin analogue) was reacted with succinic anhydride in the presence of triethylamine and 4-dimethylamino pyridine (DMAP) in 1,2-dichloroethane to provide 1,3-bis-(1,2-*O*-dimyristoyl-*sn*-glycero-3-phosphoryl)-2-succinylglycerol dimethyl ester with a 78% yield. 1,3-bis-(1,2-*O*-dimyristoyl-*sn*-glycero-3-phosphoryl)-2-succinylglycerol dimethyl ester was coupled with 4-*N*-3'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine in the presence of *N,N*-dicyclohexyl carbodimide (DCC) and DMAP in dichloromethane at room temperature for 5 hours. This reaction was followed by purification on a silica gel column (hexane-ethyl acetate, 7:3-4:1 and hexane:ethylacetate: methanol, 1:1:0.1) which afforded 4-*N*-3'-*O*-di(tert-butoxycarbonyl)-5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2-*O*-dimyristoyl-*sn*-glycero)-3-phosphoryl glycerol dimethyl ester] gemcitabine in a 72% yield. The protecting groups were removed using trifluororacetic acid (TFA) in dichloromethane at room temperature. The reaction solution was neutralized with 5% aqueous sodium bicarbonate at 0 °C, extracted with dichloromethane and concentrated. Purification of the crude compound on a silica gel column (methylene chloride-ethyl acetate, 9.8:0.2-9.6:0.4) afforded pure 5'-*O*-succinyl-[2-*O*-1,3-bis-(1,2- *O*-dimyristoyl -*sn*-glycero)-3-phosphorylglycerol dimethyl ester]gemcitabine as a viscous oil in a 50% yield. The product was characterized by ^{1}H NMR. The purity was checked by HPLC.

Example 9

Efficacy and Toxicity Studies for Gemcitabine Cardiolipin Conjugate

A. Materials

1. Chemicals

[00115] Trichloroacetic acid (TCA), sulforhodamine B (SRB), 6-[(4-Nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (NBMPR), dipyridamole, 100% ethanol and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO). Dextrose (5%) and saline was purchased from Abbott Laboratories (Abbott Park, IL).

2. Formulation preparations

[00116] Gemcitabine hydrochloride (Gemzar, Eli Lilly, Indianapolis, IN) was used within 24 hours after reconstitution. The gemcitabine-cardiolipin conjugate (Figure 18, **XXXXIII**, R & D Facility, NeoPharm Inc.,Waukegan, IL) was dispersed into 5% dextrose from an ethanol stock solution followed by 30 second vortex. The dispersion was diluted into cell culture medium for *in vitro* use or administered to animals directly. The final formulation of the gemcitabine-cardiolipin conjugate had a mean particle size of 286 nm.

3. Cell culture

[00117] Human lung A549, breast MX-1, colon HT-29 and murine leukemia P388 cell lines were obtained from the National Cancer Institute (Fredrick, MD). These cells were maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (HI-FBS). Human pancreatic BxPC-3 cancer cells were purchased from American Type Culture Center (Manassas, VA) and maintained in RPMI 1640 medium containing 10% HI-FBS, 10 mM HEPES, 1 mM sodium pyruvate and 4.5 g/L glucose. All culture media contained penicillin 100 unit/mL and streptomycin 100 μg/mL. All cells were incubated at 37 °C in a 5% CO_2 incubator. The culture media and reagents were purchased from Invitrogen Co (Carlsbad, CA).

B. Methods

1. Cytotoxicity assay

[00118] The growth inhibition was determined based on SRB assay (Chien P. *et. al. Cancer Gene Therapy* in press, 2004). In brief, different cancer cells (10,000 – 25,000 cells per well) were pre-incubated on 96-well microtiter plates overnight. Freshly prepared gemcitabine-cardiolipin conjugate suspensions or gemcitabine dilutions were added to the culture medium at final concentrations of 5×10^{-2} to 5×10^{4} nM. Cells were co-cultured with the drugs for 48 hours before fixing with TCA at 4°C for 1 hour and cytotoxicity was estimated based on total cellular protein levels as previously described (Skehan P, *et. al. J Natl Cancer Inst*, 1990, 82(13): 1107-1112). The 50% growth inhibition (GI_{50}) was estimated as the concentration of the drug that gives 50% growth inhibition. For NT inhibitor experiment, NBMPR and

dipyridamole were first dissolved in DMSO as stock solutions and further diluted in medium before use. A549 (10,000 cells/well) or BxPC-3 (20,000 cells/well) were first incubated with 1 μM NBMPR, or 10 μM dipyridamole for 1 hour. After incubating with NT inhibitors or control vehicle (DMSO), cells were further co-cultured with serial diluted gemcitabine-cardiolipin conjugate or gemcitabine for additional 48 hours. The cytotoxicity of gemcitabine-cardiolipin conjugate or gemcitabine in the presence of NT inhibitors was determined by SRB assay and compared with those without NT inhibitors. The final DMSO concentration in each well was 0.1% (v/v).

2. **Apoptosis assay**

[00119] A549 cells (10,000/well) were treated with gemcitabine-cardiolipin conjugate or gemcitabine for 48 hours as described in previous section. The cells were washed with PBS and the caspase 3/7 expression was quantified using a Caspase-Glo™ 3/7 assay kit (Promega, Madison, WI) according to the manufacturer's protocol.

3. ***In vivo* toxicity**

[00120] Multiple-dose toxicity studies were conducted on normal female CD2F1 mice (5-6 weeks old). Mice were randomized into groups of 5 based on body weight and were injected with gemcitabine-cardiolipin conjugate or gemcitabine at doses of 18, 27 and 36 μmol/kg for 5 consecutive days. Mice in control group were given equal dose volume of vehicle (5% dextrose and 5% ethanol). The toxicities were evaluated by the mortality, body weight loss and by obtaining the peripheral blood cell counts and plasma levels of alanine transaminase (ALT), aspartate transaminase (AST) at the end of the study. Blood samples were collected from the orbital sinus into either K_2-DETA-coated tubes for CBC or plasma separating tubes for plasma enzyme and creatinine analysis. All laboratory examinations were conducted at Antech Diagnostics (Chicago, IL). Percentage of body weight change was calculated as $100 \times (W_t-W_0)/W_t$; where W_t represents the body weight at any time point and W_0 represent initial point.

4. **Tumor models**

[00121] A mouse leukemic model was established with female CD2F1 mice (5-6 weeks old). Each mouse was injected with log-phase P388 cells (1×10^5 cells in 0.2 ml PBS) via lateral tail veins on day 0. Next day, mice were randomly divided into groups of 5 based on their body weight and treated with gemcitabine-cardiolipin conjugate or gemcitabine at doses of 9, 18 and 27 μmol/kg. Mice, in the control group, were administrated with a control vehicle (5% dextrose and 5% ethanol). Mice were observed, once a day, for signs of moribundity and mortality for 60 days. Body weights were recorded during dosing and twice a week thereafter.

[00122] A human pancreatic tumor xenograft was established with female CB-17 SCID mice (4-5 weeks old) by s.c. injection of 2×10^6 BxPC-3 cells at right flank region. Mice were treated with gemcitabine-cardiolipin conjugate, gemcitabine or control vehicle intravenously when the tumor reached a volume of 80-160 mm^3. Tumor length (L) and width (W) were measured with a digital caliper twice a week. The tumor volume (V) and tumor growth (% of initial tumor volume) were calculated as follows: V (mm^3) = length $\times$ (width/2)^2 $\times$ π; where $\pi = 3.14$; Tumor growth or % initial tumor volume = 100 $\times$ (V_1/V_0); where V_1 represents the tumor volume at any given day and V_0 is the tumor volume on the day of treatment initiation.

5. **Statistical comparison**

[00123] Data were expressed as mean ± SD. Statistical comparisons were made by use of the Student *t* test ($P < 0.05$).

C. Results

1. Gemcitabine-cardiolipin conjugate-induced cytotoxicity in cancer cells

[00124] Both the gemcitabine-cardiolipin conjugate and gemcitabine showed dose-dependent cytotoxicity against human pancreatic (BxPC-3), lung (A549), breast (MX-1), colon (HT-29) cancer cells and murine leukemia (P388) after 48 hrs treatment (Figure 19). At higher concentration (≥ 5 μM), cytolysis was found in cells treated with the gemcitabine-cardiolipin conjugate. A similar phenomenon did not occur with cells treated with gemcitabine. GI_{50} was estimated from the dose curves in Figure 19a and summarized in Table 1. Among the cell lines tested, P388 showed the highest sensitivity to the gemcitabine-cardiolipin conjugate with a GI_{50} of 44 nM followed by HT-29 and BxPC-3 with GI_{50} of 69 and 182 nM, respectively. BxPC-3 and P388 cell lines were chosen for further *in vivo* efficacy evaluation.

Cell lines	Gemcitabine-cardiolipin conjugate (nM)	Gemcitabine (nM)	Change in Sensitivity GI_{50} (fold)
BxPC-3	182	19	10
A549	589	95	6
MX-1	575	205	3
HT-29	69	4	17
P388	44	8	6

Table 1 GI_{50} of gemcitabine-cardiolipin conjugate and Gemcitabine on Different Cancer Cell Lines. Cells were incubated with serial diluted gemcitabine-cardiolipin conjugate or gemcitabine for 48 hrs before measurement of total cellular level with SRB assay.

[00125] The time-dependent cytotoxicity of gemcitabine-cardiolipin conjugate and gemcitabine was also investigated. The onset of gemcitabine-cardiolipin conjugate effects, on cancer cell lines, was faster compared to that of gemcitabine. The cytotoxicity could be detected as early as 5 hours after the treatment with 50 μM of gemcitabine-cardiolipin conjugate in A549 cells (Figure 19b). In contrast, gemcitabine-induced cytotoxicity did not reach significant levels until 24 hrs after the treatment. These results indicate that the gemcitabine-cardiolipin conjugate enters the cells and exerts its cytotoxicity more rapidly than gemcitabine. At 5 μM, both the gemcitabine-cardiolipin conjugate and gemcitabine showed similar time-dependent cytotoxicity against A549 cells (Figure 19b).

[00126] Apoptosis has been shown to be one of the major mechanisms of gemcitabine-induced cytotoxicity (Nabhan C. *et. al.* , *Mol Cancer Ther.*, 2001, 1(13): 1221-1227). To investigate whether the conjugation of cardiolipin has modified the cytotoxicity pathway of gemcitabine, apoptosis was determined in A549 cells treated with gemcitabine-cardiolipin conjugate and gemcitabine by detecting the increase of caspase 3/7 expression. As shown in Figure 20, both the gemcitabine-cardiolipin conjugate and gemcitabine enhanced caspase 3/7 expression in A549 cells as compared to untreated cells. The signal of caspase 3/7 increased as the concentration of either the gemcitabine-cardiolipin conjugate or gemcitabine increased. This indicated that both drugs induced apoptosis by activating caspase cascade.

2. Equilibrative NT inhibitor study

[00127] NT is one main factor in determining the gemcitabine sensitivity in cancer chemotherapy. Among all the members from NT family, equilibrative NT is most important because of its broad tissue expression (Cass C.E., *et. al. Pharm Biotechnol*, 1999, 12: 313-352).

[00128] Tumor cells with non-functioning NT are resistant to gemcitabine (Achiwa H. *et. al. Cancer Sci*, 2004, 95(9): 753-757; Mackey J.R. *et. al.*, *Cancer Res*, 1998, 58(19): 4349-4357). In order to explore the potential application of gemcitabine-cardiolipin conjugate on NT-deficient and gemcitabine-resistant tumors, we investigated the cytotoxicity of gemcitabine-cardiolipin conjugates against A549 cells in the presence of dipyridamole. By incubating with 10 μM of dipyridamole, A549 cells with non-functioning NT were obtained (Mackey J.R. *et. al.*, *Cancer Res*, 1998, 58(19): 4349-4357). Blocking the normal function of NT, by dipyridamole, had no impact on gemcitabine-cardiolipin conjugate -induced cytotoxicity against A549 cells as indicated by insignificant changes in its growth inhibition curves (Figure 21a). In contrast, when NT was blocked with dipyridamole, the growth curve was shifted toward right, an indication of decreased cytotoxicity of gemcitabine against A549 cells (Figure 21b). The same experiment was also conducted with BxPC-3 cells. The potency of dipyridamole on gemcitabine-cardiolipin conjugate or

gemcitabine-induced cytotoxicity against A549 and BxPC-3 cells was expressed with a ratio of GI_{50} in the presence and absence of dipyridamole (Table 2).

	Gemcitabine-cardiolipin conjugate			Gemcitabine		
Dipyridamole (10 μM)	(-)	(+)	Ratio (+) / (-)	(-)	(+)	Ratio (+) / (-)
A549	1190	273	0.23	123	6262	51
BxPC-3	649	429	0.66	128	3880	30

Table 2 Effect of Dipyridamole on GI_{50} (nM) of Gemcitabine-cardiolipin conjugate or Gemcitabine against A549 and BxPC-3 Cells. Cells were treated with serial diluted gemcitabine-cardiolipin conjugate or gemcitabine for 48 hrs in the presence or absence of 10 μM of dipyridamole before SRB assay.

3. ***In vivo* toxicity study**

[00129] Based on our preliminary results, three doses of 18, 27 and 36 μmol/kg were used to find the maximum tolerable toxicity of gemcitabine and the gemcitabine-cardiolipin conjugate in CD2F1 mice. After 6 daily injections, all mice treated with gemcitabine at 27 and 36 μmol/kg were found moribund and had to be sacrificed on day 7. Mice treated with gemcitabine at 18 and gemcitabine-cardiolipin conjugate at 36 μmol/kg developed dehydration and rough coat but were not considered moribund. Therefore, the maximum tolerable dose of gemcitabine, at the current schedule (6 daily injections) on CD2F1 mice, was considered to be 18 μmol/kg for gemcitabine and 36 μmol/kg for the gemcitabine-cardiolipin conjugate.

[00130] The toxicity of gemcitabine-cardiolipin conjugate at 18 μmol/kg after 6 daily treatments and the body weight loss on Day 7 was significantly less compared to gemcitabine. When mice were treated with gemcitabine-cardiolipin conjugate at 18 μmol/kg for 5 days, the maximum body weight loss was only 3% compare to 22% for gemcitabine. In addition, a significant decrease in WBC was found in mice treated with gemcitabine (Table 3) but not in mice treated with the gemcitabine-cardiolipin conjugate. Both the gemcitabine-cardiolipin conjugate and gemcitabine-treated mice developed neutropenia. There were no significant differences in RBC, HGB, platelet counts, plasma ALT, AST and creatine levels in mice treated with the gemcitabine-cardiolipin conjugate as compared to the control vehicle, indicating no significant liver or kidney toxicity of gemcitabine-cardiolipin conjugate. These results were confirmed by histopathologic examination where the gemcitabine-cardiolipin conjugate -treated mice did not induce any clinically significant changes in morphology of lung, liver or kidney.

	Control	Gemzar 18 μmol/kg	Gemcitabine-cardiolipin conjugate 18 μmol/kg
% Max changes in body weight after 6 injections	1.9 ± 6.0	-23.8 ± 8.8	-11.0 ± 3.7
WBC ($10^3/\mu l$)	3.4 ± 0.9	1.6 ± 0.4	3.5 ± 1.2
RBC ($10^6/\mu l$)	10.7 ± 0.4	10.9 ± 0.6	11.3 ± 0.4
HGB (g/dL)	16.3 ± 1.1	16.8 ± 0.4	17.6 ± 0.7
Neutrophils ($/\mu l$)	238 ± 56	27 ± 25	71 ± 47
Lymphocytes ($10^3/\mu l$)	2.8 ± 1.1	1.5 ± 0.3	2.8 ± 0.7
Platelet ($10^3/\mu l$)	513 ± 64	452 ± 88	417 ± 120
Total protein (g/dL)	6.4 ± 0.4	4.8 ± 1.1	6.6 ± 0.5
Albumin (g/dL)	4.3 ± 0.5	2.9 ± 0.8	4.1 ± 0.6
AST	109.4	152.4	155.6

Table 3 Body Weight Changes and Hematology Test in Mice Treated with 6 Daily Doses of Gemcitabine-cardiolipin conjugate or Gemcitabine

4. ***In vivo* efficacy studies**

[00131] A systemic leukemia model was established by *i.v.* injection of P388 murine leukemia cells (1×10^5) to female CD2F1 mice. Without treatment, the mice died after an average of 12 days. Treatment with the gemcitabine-cardiolipin conjugate at doses of 18 or 27 μmol/kg body weight for 5 days increased median survival time by 55 to 73% (Figure 22), suggesting a strong anti-leukemic activity from gemcitabine-cardiolipin conjugate. A dose of 18 μmol/kg, gemcitabine resulted in the toxic death of all treated mice. At 9 μmol/kg, gemcitabine exhibited comparable survival increase to the gemcitabine-cardiolipin conjugate at 18 μmol/kg. It was noted that 25% of mice treated with the gemcitabine-cardiolipin conjugate at 18 or 27 μmol/kg survived for more than 60 days.

[00132] For the human pancreatic (BxPC-3) xenograft model, the mice were initially treated with 4 weekly injections and 3 twice a week injections of the gemcitabine-cardiolipin conjugate at doses of 18 and

36 μmol/kg or gemcitabine at a dose of 18 μmol/kg. As indicated in Figure 23, the growth of established BxPC-3 tumor cells was significantly inhibited by treatment with the gemcitabine-cardiolipin conjugate or gemcitabine at a dose of 18 μmol/kg. On day 50, the growth inhibition by the gemcitabine-cardiolipin conjugate was 52% ($P = 0.0001$) and 32% ($P = 0.0002$) by gemcitabine. At 36 μmol/kg, the gemcitabine-cardiolipin conjugate inhibited the growth by 64% ($P = 0.0001$) (Figure 23).

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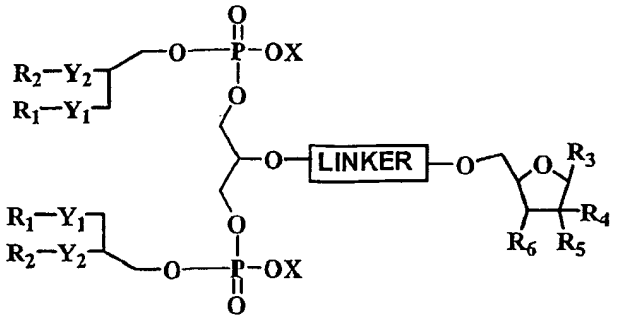
WHAT IS CLAIMED IS:

1. A nucleoside-cardiolipin conjugate having the following structure:



I

wherein Y_1 and Y_2 are the same or different and are –O-C(O)-, -O-, -S-, -NH-C(O)- or the like;

R_1 and R_2 are the same or different are selected from a group consisting of H, saturated alkyl group and unsaturated alkyl group;

X is selected from a group consisting of H, alkyl group and a cation;

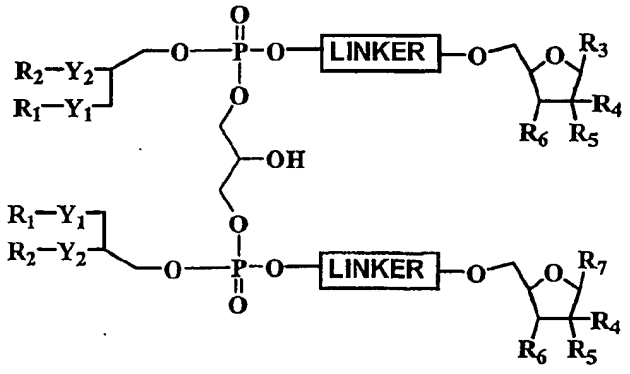
R_3 is a nucleoside selected from a group consisting of cytosine, guanine, adenine, thymine, uracil, inosine, xanthine and hypoxanthine;

R_4 and R_5 are the same or different and are selected from a group consisting of hydrogen, hydroxyl, halo group, nitro, alkyl group, substituted alkyl and alkoxy group;

R_6 is selected from a group consisting of hydrogen, hydroxyl group, azido group, amino group, alkyl group, halo group and substituted amino;

Five membered cyclic sugar is selected from a group consisting of ribofuranose, arabinofuranose, deoxyribofuranose and xylofuranose.

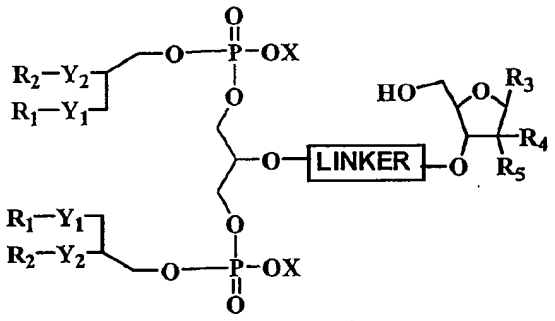
2. A nucleoside-cardiolipin conjugate having the following structure:



II

wherein Y_1 and Y_2 are the same or different and are –O-C(O)-, -O-, -S-, -NH-C(O)- or the like;
R_1 and R_2 are the same or different are selected from a group consisting of H, saturated alkyl group and unsaturated alkyl group;
R_3 and R_7 are nucleosides selected from a group consisting of cytosine, guanine, adenine, thymine, uracil, inosine, xanthine and hypoxanthine;
R_4 and R_5 are the same or different and are selected from a group consisting of hydrogen, hydroxyl, halo group, nitro, alkyl group, substituted alkyl and alkoxy group;
R_6 is selected from a group consisting of hydrogen, hydroxyl group, azido group, amino group, alkyl group, halo group and substituted amino;
Five membered cyclic sugar is selected from a group consisting of ribofuranose, arabinofuranose, deoxyribofuranose and xylofuranose.

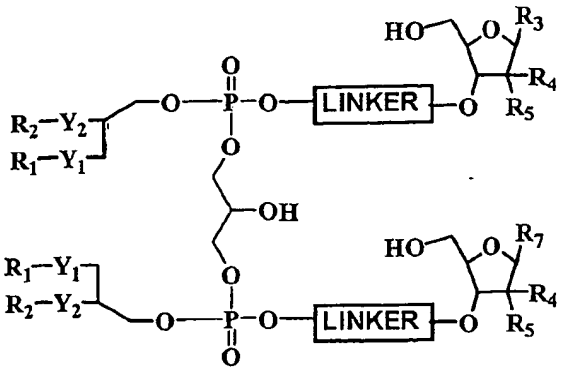
3. A nucleoside-cardiolipin conjugate having the following structure:



III

wherein Y_1 and Y_2 are the same or different and are –O-C(O)-, -O-, -S-, -NH-C(O)- or the like;
R_1 and R_2 are the same or different are selected from a group consisting of H, saturated alkyl group and unsaturated alkyl group;
X is selected from a group consisting of H, alkyl group and a cation;
R_3 is a nucleoside selected from a group consisting of cytosine, guanine, adenine, thymine, uracil, inosine, xanthine and hypoxanthine;
R_4 and R_5 are the same or different and are selected from a group consisting of hydrogen, hydroxyl, halo group, nitro, alkyl group substituted alkyl and alkoxy group;
Five membered cyclic sugar is selected from a group consisting of ribofuranose, arabinofuranose, deoxyribofuranose and xylofuranose.

4. The nucleoside-cardiolipin conjugate having the following structure:



IV

wherein Y_1 and Y_2 are the same or different and are –O-C(O)-, -O-, -S-, -NH-C(O)- or the like;

R_1 and R_2 are the same or different are selected from a group consisting of H, saturated alkyl group and unsaturated alkyl group;

R_3 and R_7 are nucleosides selected from a group consisting of cytosine, guanine, adenine, thymine, uracil inosine, xanthine and hypoxanthine;

R_4 and R_5 are the same or different and are selected from a group consisting of hydrogen, hydroxyl, halo group, nitro, alkyl group, substituted alkyl and alkoxy group;

Five membered cyclic sugar is selected from a group consisting of ribofuranose, arabinofuranose, deoxyribofuranose and xylofuranose.

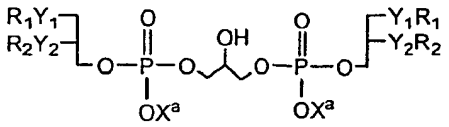
5. The composition of claim 1 or 3, wherein the X is an alkyl group ranging from $C_1 - C_{10}$.
6. The composition of claim 1 or 3, wherein X is a non-toxic cation.
7. The composition of claim 6, wherein the non-toxic cation is selected from a group consisting of ammonium, sodium, potassium, calcium and barium.
8. The composition of any of claims 1-4, wherein R_3 is optionally substituted with substituents selected from the group consisting of halo, nitro, alkyl, alkenyl, alkoxy, aryl, trifluoromethyl and $N(R^a)(R^b)$ wherein R^a and R^b are independently selected from a group consisting of H and alkyl groups.
9. The composition of claim 2 or 4, wherein R_7 is optionally substituted with substituents selected from the group consisting of halo, nitro, alkyl, alkenyl, alkoxy, aryl, trifluoromethyl and $N(R^a)(R^b)$ wherein R^a and R^b are independently selected from a group consisting of H and alkyl groups.
10. The composition of any of claims 1-9, wherein the nucleoside is a β-nucleoside.
11. The composition of any of claims 1-9, wherein the nucleoside is an α-nucleoside.
12. The composition of any of claims 1-11, wherein the linker comprises a first end and a second end wherein said first end is attached to a lipid through a first linker functional group and said second end is attached to a nucleoside through a second linker functional group.

13. The composition of claim 12, wherein the first and second linker functional groups are selected from a group consisting of hydroxyl group, primary amino group, secondary amino group, phosphate group or substituted derivative thereof, carboxylic acid, carbonate, carbonyl and carbamate group.
14. The composition of any of claims 12-13, wherein the linker further comprises $(CH_2)_n$, wherein n=0-20.
15. The composition of claim 14, wherein the $(CH_2)_n$ is substituted with functional groups selected from a group consisting of alkyl, alkoxy, hydroxyl, carbonyl, carboxyl, carbamate, aldehyde, amino, halo, polyalkoxy, PEG group, phosphate, phosphonate and pyrophosphate.
16. The composition of any of claims 12-13, wherein the linker is selected from a group consisting of an alkyl, substituted alkyl, dicarbonyl alkyl, alkoxy, polyalkoxy, PEGylated group, phosphate, phosphonate, diphosphate, triphosphate, phosphodiester, phosphotriester, phosphoramidite, peptide, dipeptide and polypeptide.
17. The composition of claim 1, wherein R_3 is cytosine; R_4 and R_5 are fluoro; R_6 is hydroxyl group; X is selected from a group consisting of methyl or ammonium; Y_1 and Y_2 are oxo (-O-) groups; the linker is succinimidoyl group and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
18. The composition of claim 17, wherein R_1 is H, C_1-C_{34} saturated or unsaturated alkyl groups and R_2 is a methyl group.
19. The composition of claim 1 wherein R_3 is cytosine; R_4 and R_5 are fluoro; R_6 is hydroxyl group; X is methyl or ammonium; Y_1, Y_2 are -O-C(O)-; the linker is a succinimidoyl group; R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
20. The composition of claim 3, wherein R_3 is cytosine; R_4 and R_5 are fluoro groups; X is methyl or ammonium; Y_1, Y_2 are oxo (-O-) groups;the linker is succinimidoyl group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
21. The composition of claim 20, wherein R_1 is H, C_1-C_{34} saturated or unsaturated alkyl groups and R_2 is a methyl group.
22. The composition of claim 3, wherein R_3 is cytosine, R_4 and R_5 are fluoro group, X is methyl or ammonium, Y_1, Y_2 are -O-(CO)- groups, the linker is a succinimidoyl group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
23. The composition of claim 1, wherein R_3 is cytosine, R_4 and R_5 are fluoro, R_6 is hydroxyl group, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, and the linker is a phosphodiester or phosphotriester group and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
24. The composition of claim 23, wherein R_1 is H, C_1-C_{34} saturated or unsaturated alkyl groups, and R_2 is methyl group.

25. The composition of claim 1, wherein R_3 is cytosine, R_4 and R_5 are fluoro, R_6 is hydroxyl group, X and R_8 are methyl or ammonium, Y_1, Y_2 are –O-(CO)- groups, and the linker is a phosphodiester or phosphotriester group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
26. The composition of claim 3, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) groups, the linker is a phosphodiester or phosphotriester group and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
27. The composition of claim 26, wherein R_1 is H, C_1-C_{34} is saturated or unsaturated alkyl groups, and R_2 is a methyl group.
28. The composition of claim 3, wherein R_3 is cytosine, R_4 and R_5 are fluoro groups, X and R_8 are methyl or ammonium, Y_1, Y_2 are -O-(CO)- groups, and the linker is a phosphodiester or phosphotriester group and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
29. The composition of claim 2, wherein R_3 and R_7 are cytosine, R_4 and R_5 are fluoro, R_6 is hydroxyl, Y_1, Y_2 are oxo (-O-) or –O-C(O)- groups, the linker is $-CH_2CH_2CH_2NHCOCH_2CH_2C(O)$-group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups
30. The composition of claim 4, wherein R_3 and R_7 are cytosine, R_4 and R_5 are fluoro, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is $-CH_2CH_2CH_2NHCOCH_2CH_2C(O)$-group, R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
31. The composition of claim 1, wherein R_3 is cytosine, R_4 and R_6 are hydroxyl, R_5 is hydrogen, X is methyl or ammonium, Y_1, Y_2 are oxo (-O-) or –O-C(O)- groups, and the linker is succinimidoyl group, R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
32. The composition of claim 3, wherein R_3 is cytosine, R_4 is hydroxyl, R_5 is hydrogen, X is methyl or ammonium, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is succinimidoyl group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
33. The composition of claim 1, wherein R_3 is cytosine, R_4 and R_6 are hydroxyl, R_5 is hydrogen, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) or –O-C(O)- groups, the linker is a phosphodiester or phosphotriester group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
34. The composition of claim 3, wherein R_3 is cytosine, R_4 is hydroxyl and R_5 is hydrogen, X and R_8 are methyl or ammonium, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is a phosphodiester or phosphotriester group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
35. The composition of claim 2, wherein R_3 and R_7 are cytosine, R_4 and R_6 are hydroxyl, R_5 is hydrogen, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is –

$CH_2CH_2CH_2NHCOCH_2CH_2C(O)$-group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.

36. The composition of claim 4, wherein wherein R_3 and R_7 are cytosine, R_4 and R_6 are hydroxyl, R_5 is hydrogen, Y_1, Y_2 are oxo (-O-) or -O-C(O)- groups, the linker is – $CH_2CH_2CH_2NHCOCH_2CH_2C(O)$-group, and R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups.
37. A composition of any of claims 1-36, wherein the nucleoside-cardiolipin conjugate is a prodrug.
38. A pharmaceutical composition comprising the nucleoside-cardiolipin conjugate of any of claims 1-37 and a pharmaceutically acceptable carrier.
39. A method of preparing a nucleoside-cardiolipin conjugate comprising reacting a cardiolipin derivative **XXXXI** with succinic anhydride in an inert solvent in the presence of base.



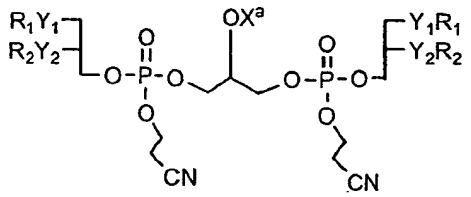
XXXXI

Wherein X^a is a protecting group;
R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups;
Y_1 and Y_2 are oxo(-O-) or –O-C(O)- groups.

40. The method of claim 39, wherein X^a is selected from a group consisting of a phosphate, methyl group, benzyl group, 2-cyanoethyl, ethyl.
41. The method of claim 39-40, further comprising the addition of a gemcitabine derivative in the presence of dicyclohexylcarbodimide (DCC), *N'N'*-dimethyaminopyridine (DMAP) and an inert solvent.
42. The method of claim 41, wherein the gemcitabine derivative is selected from a group consisting of 4-*N*-3'-*O*-Bis(X^b)gemcitabine and 4-*N*-5'-*O*-Bis(X^b)gemcitabine, wherein X^b is a protecting group.
43. The method of claim 42, wherein X^b is selected from a group consisting of benzyloxycarbonyl, trityl, tert-butoxycarbonyl, benzyloxymethyl, trimethylsilyl, tert-butyldimethylsilyl, benzoyl, acetyl, pivaloyl and levulinoyl.
44. The method of claim 39-43, further comprising the removal of the protecting groups.
45. The method of claim 44, wherein the removal of the protecting groups comprises the addition of an acidic medium in an inert solvent.
46. The method of claim 45, wherein the acidic medium is TFA and the inert solvent is dichloromethane.

47. The method of claim 44, wherein the removal of the protecting groups comprises catalytic hydrogenation.
48. The method of claim 44, wherein the removal of protecting groups comprises heating sodium iodide in the presence of 2-butanone or acetone.
49. The method of any of claims 39-48, further comprising the addition of dilute ammonium hydroxide.
50. The method of any of claims 39-40, further comprising the addition of *N,N,*-diisopropylmethylphosphonamidic chloride in the presence of a base in an inert solvent.
51. The method of claim 50, further comprising the addition of a gemcitabine derivative in the presence of dicyclohexylcarbodimide (DCC), *N'N'*-dimethyaminopyridine (DMAP) and an inert solvent.
52. The method of claim 51, wherein the gemcitabine derivative is selected from a group consisting of 4-*N*-3'-*O*-Bis(X^b)gemcitabine and 4-*N*-5'-*O*-Bis(X^b)gemcitabine, wherein X^b is a protecting group.
53. The method of claim 52, wherein X^b is selected from a group consisting of benzyloxycarbonyl, trityl, tert-butoxycarbonyl, benzyloxymethyl, trimethylsilyl, tert-butyldimethylsilyl, benzoyl, acetyl, pivaloyl and levulinoyl.
54. The method of claim 50-53, further comprising removal of the protecting groups.
55. The method of claim 54, wherein the removal of the protecting groups comprises the addition of an acidic medium in an inert solvent.
56. The method of claim 55, wherein the acidic medium is TFA and the inert solvent is dichloromethane.
57. The method of claim 54, wherein the removal of the protecting groups comprises catalytic hydrogenation.
58. The method of claim 54, wherein the removal of protecting groups comprises heating sodium iodide in the presence of 2-butanone or acetone.
59. The method of any of claims 50-58, further comprising the addition of dilute ammonium hydroxide.
60. The method of any of claims 39-40, further comprising the addition of benzyl tetraisopropylphosphoramidite in the presence of 1H-tetrazole.
61. The method of claim 60, further comprising the addition of a gemcitabine derivative in the presence of dicyclohexylcarbodimide (DCC), *N'N'*-dimethyaminopyridine (DMAP) and an inert solvent.
62. The method of claim 61, wherein the gemcitabine derivative is selected from a group consisting of 4-*N*-3'-*O*-Bis(X^b)gemcitabine and 4-*N*-5'-*O*-Bis(X^b)gemcitabine, wherein X^b is a protecting group.

63. The method of claim 62, wherein X^b is selected from a group consisting of benzyloxycarbonyl, trityl and tert-butoxycarbonyl, benzyloxymethyl, trimethylsilyl, tert-butyldimethylsilyl, benzoyl, acetyl, pivaloyl, levulinoyl.
64. The method of any of claims 60-63, further comprising oxidation with a suitable oxidizing agent.
65. The method of any of claims 60-64, further comprising removal of the protecting groups.
66. The method of claim 65, wherein the removal of the protecting groups comprises the addition of an acidic medium in an inert solvent.
67. The method of claim 66, wherein the acidic medium is TFA and the inert solvent is dichloromethane.
68. The method of claim 65, wherein the removal of the protecting groups comprises catalytic hydrogenation.
69. The method of claim 65, wherein the removal of protecting groups comprises heating sodium iodide in the presence of 2-butanone or acetone.
70. The method of any of claims 60-69 further comprising the addition of dilute ammonium hydroxide.
71. A method of preparing a nucleoside-cardiolipin conjugate comprising hydrogenation of a cardiolipin derivative **XXXXII**.



XXXXII

Wherein X^a is a protecting group;

R_1 and R_2 are the same or different and are H, C_1-C_{34} saturated or unsaturated alkyl groups;

Y_1 and Y_2 are oxo(-O-) or –O-C(O)- groups.

72. The method of claim 71, wherein X^a is selected from a group consisting of benzyl, ter-butyldimethylsilyl, levulinoyl, pivaloyl, methoxymethyl and benzyloxymethyl.
73. The method of any of claims 71-72, further comprising the addition of succinic anhydride.
74. The method of any of claims 71-73, further comprising the addition of a gemcitabine derivative.
75. The method of claim 74, wherein the gemcitabine derivative is selected from a group consisting of 4-*N*-3'-*O*-Bis(X^b)gemcitabine and 4-*N*-5'-*O*-Bis(X^b)gemcitabine, wherein X^b is a protecting group.

76. The method of claim 75, wherein X^b is selected from a group consisting of benzyloxycarbonyl, trityl, tert-butoxycarbonyl, benzyloxymethyl, trimethylsilyl, tert-butyldimethylsilyl, benzoyl, acetyl, pivaloyl and levulinoyl.
77. The method of any of claims 71-76, further comprising the removal of protecting groups.
78. The method of claim 77, wherein the removal of the protecting groups comprises the addition of an acidic medium in an inert solvent.
79. The method of claim 78, wherein the acidic medium is TFA and the inert solvent is dichloromethane.
80. A nucleoside-cardiolipin conjugate prepared in accordance with the method of any of claims 39-49.
81. A nucleoside-cardiolipin conjugate prepared in accordance with the method of any of claims 50-59.
82. A nucleoside-cardiolipin conjugate prepared in accordance with the method of any of claims 60-70.
83. A nucleoside-cardiolipin conjugate prepared in accordance with the method of any of claims 71-79.
84. A method for preparing a liposome, comprising preparing a nucleoside-cardiolipin by any of the methods of claims 39-79 and then including said nucleoside-cardiolipin in a liposome.
85. A method of retaining an active agent in a liposome, comprising preparing a nucleoside-cardiolipin or a cardiolipin analogue by any of the methods of claims 39-79 and then including said nucleoside-cardiolipin analogue and at least one active agent in a liposome.
86. The method of claim 85, wherein the active agent is a drug.
87. The method of claim 85, wherein the active agent is selected from a group consisting of antineoplastic, antifungal and antibiotic agents.
88. The method of claim 85, wherein the active agent includes an oligonucleotide.
89. The method of any of claims 85-88, wherein the active agent becomes entrapped within the liposomes.
90. The method of any of claims 85-88, wherein the active agent becomes complexed with the nucleoside-cardiolipin analogue.
91. The method of any of claims 84-90, further comprising lyophilizing the liposomes.
92. The method of any of claims 84-91, further comprising the addition of a hydrophilic mixture.

93. A liposomal composition prepared in accordance with the method of any of claims 84-92.
94. A composition comprising the nucleoside-cardiolipin conjugate of any claims 1-37.
95. The composition of claim 94, further comprising a phosphatidylcholine.
96. The composition of any of claims 94-95, further comprising a sterol.
97. The composition of any of claims 94-96, further comprising a tocopherol.

98. The composition of claim 94, further comprising phosphatidylcholine, sterol and α-tocopherol.
99. The composition of any of claims 93-98, further comprising at least one lipid selected from a group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, cholesterol, α-tocopherol, natural or synthetic cardiolipin and analogues thereof, synthetic cationic cardiolipin analogues and derivatives thereof, phosphatidylserine, phosphatidylinositol, sphingomyelin, lysophosphotidylglycerol, lysophosphatidic acid, lysophosphotidylcholine, and lysophosphatidylserine.
100. The composition of any of claims 93-99, wherein the lipids are pegylated.
101. The composition any of claims 93-99, wherein the lipids are linked to polyethylene glycol derivatives.
102. The composition of any of claims 93-101, further comprising a targeting agent.
103. The composition of claim 102, wherein the targeting agent is a protein.
104. The composition of claim 103, wherein the protein is selected from a group consisting of antibodies, antibody fragments, peptides, peptide hormones, receptor ligands and mixtures thereof.
105. The composition of any of claims 93-104, further comprising a pharmaceutically acceptable carrier.
106. The composition of any of claims 93-105, wherein said liposomes comprise vesicles having a size of about 5 μm or less.
107. The composition of claim 106, wherein said liposomes comprise vesicles having a size of about 1 μm or less.
108. The composition of claim 107, wherein said liposomes comprise vesicles having a size of about 0.5 μm or less.
109. The composition of claim 108, wherein said liposomes comprise vesicles having a size of about 0.1 μm or less.
110. The composition of any of claims 93-109, wherein said liposome is a mixture of multilamellar vesicles and unilamellar vesicles.
111. The composition of any of claims 93-109, wherein said liposome is comprised of multilamellar vesicles.
112. The composition of any of claims 93-109, wherein said liposome is comprised of unilamellar vesicles.

113. A method for the treatment of a cellular proliferative disease comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.
114. A method for the alleviation of a cellular proliferative disease comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.

115. A method for the treatment of a cellular proliferative disease comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

116. A method for the alleviation of a cellular proliferative disease comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

117. The method of any of claims 113-116, wherein said patient is human.

118. The method of any of claims 113-117, wherein said cellular proliferative disease is cancer.

119. The method of claim 118, wherein the cancer is selected from a group consisting of cancers of the head, neck, brain, blood, breast, lung, pancreas, bone, spleen, bladder, prostate, testes, colon, kidney ovary and skin.

120. The method of any of claims 113-119, wherein the composition is administered adjunctively with a second antineoplastic agent.

121. The method of any of claims 113-119, wherein the composition is administered prior to, concurrently with, or after the second antineoplastic agent.

122. The method of any of claim 120-121, wherein the second antineoplastic agent is radiation.

123. The method of any of claims 120-121, wherein the second antineoplastic agent is chemotherapy.

124. A method for the treatment of a viral disease comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.

125. A method for the alleviation of a viral disease comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.

126. A method for the treatment of a viral disease comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

127. A method for the alleviation of a viral disease comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

128. The method of any of claims 124-127, wherein said patient is human.

129. The method of any of claims 124-128, wherein the viral disease is selected from a group consisting of HIV, herpes simplex viruses, human herpes virus 6, human herpes virus 7, human herpes virus 8, orthopoxviruses, ebola virus, influenza virus, tuberculosis, hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E, hepatitis G, parainfluenza virus, respiratory syncytial virus,cholera, pneumonia, SARS virus, canary virus, West Nile virus (WNV), respiratory syncytial virus (RSV), dengue virus, vericella zoster virus, corona viruses, vaccinia virus, cytomegalovirus (CMV), human rhinovirus (HRV), papilloma virus (PV) and Epstein Barr virus.

130. A method for the alleviation of a bone disorder comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.

131. A method for the treatment of a bone disorder comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.

132. A method for the alleviation of bone resorption comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.

133. A method for increasing bone formation comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.

134. A method for increasing bone mass comprising administering a therapeutically effective amount of the composition of any of claims 1-38 to a patient in need thereof.

135. A method for the alleviation of a bone disorder comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

136. A method for the treatment of a bone disorder comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

137. A method for the alleviation of bone resorption comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

138. A method for increasing bone formation comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

139. A method for increasing bone mass comprising administering a therapeutically effective amount of the composition of any of claims 93-112 to a patient in need thereof.

140. The method of any of claims 130-139, wherein said patient is human.

141. The method of any of claims 130-140, wherein the bone disorder is selected from a group consisting of osteoporosis, Paget's disease, metastatic bone cancers, hyperparathyroidism, rheumatoid arthritis, algodistrophy, sterno-costo-clavicular hyperostosis, Gaucher's disease, Engleman's disease.

142. The method of any of claims 130-141, wherein the composition is administered adjunctively with a second therapeutic agent.

143. The method of any of claims 130-141, wherein the composition is administered prior to, concurrently with, or after the second therapeutic agent.

144. The method of any of claims 113-143, wherein the administration is intravaneously.

145. The method of any of claims 113-143, wherein the administration is dermally.

146. The method of any of claims 113-143, wherein the administration is directly to a tumor.

147. The method of any of claims 113-143, wherein the administration is orally.

148. A kit for administering the liposomal composition of any of claims 1-38 to a mammal.

149. A kit for administering the liposomal composition of any of claims 93-112 to a mammal.

150. The kit of claim 148, wherein the kit comprises the composition of any of claims 1-38 and an instructional manual which describes the administration of the composition to the mammal.

151. The kit of claim 149, wherein the kit comprises the composition of any of claims 93-112 and an instructional manual which describes the administration of the composition to the mammal.

152. The kit of any of claims 148-151, further comprising a sterile solvent suitable for dissolving or suspending the composition prior to administering the composition to the mammal.

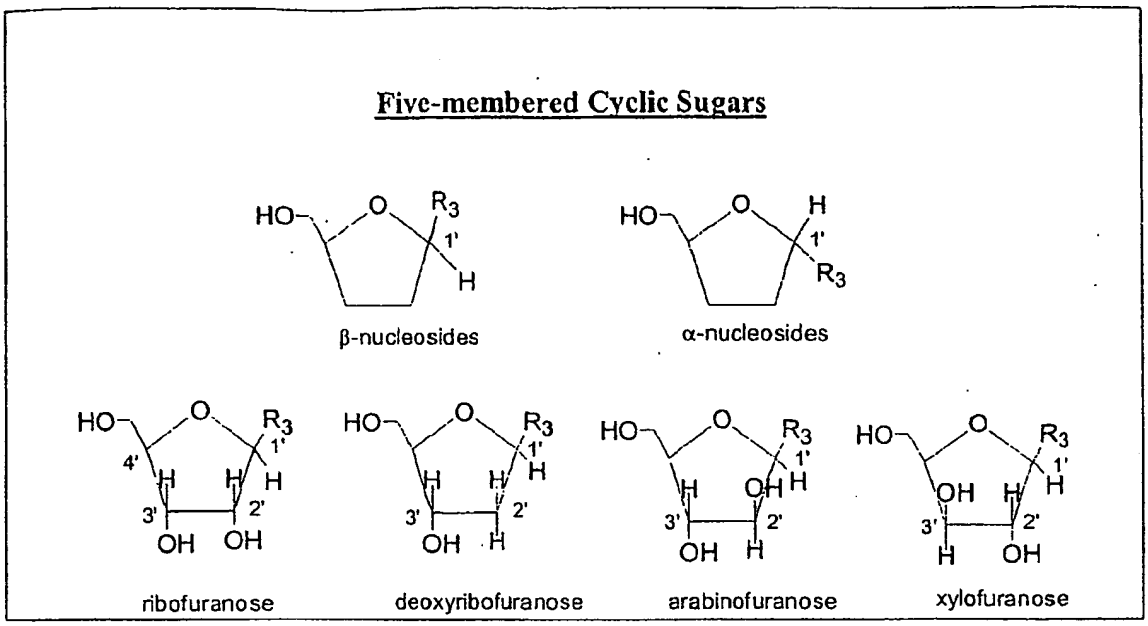

Five-membered Cyclic Sugars
β-nucleosides
α-nucleosides
ribofuranose
deoxyribofuranose
arabinofuranose
xylofuranose

Fig. 1

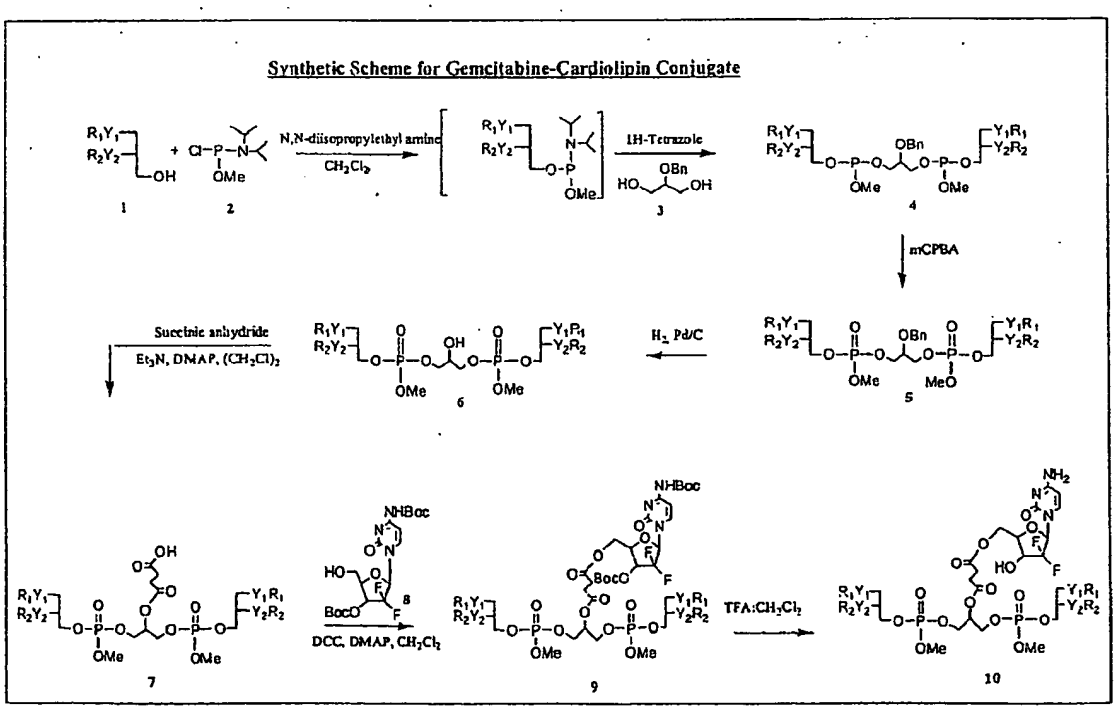

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
N,N-diisopropylethyl amine
CH2Cl2
1H-Tetrazole
mCPBA
Succinic anhydride
Et3N, DMAP, (CH2Cl)2
H2, Pd/C
DCC, DMAP, CH2Cl2
TFA:CH2Cl2

Fig. 2

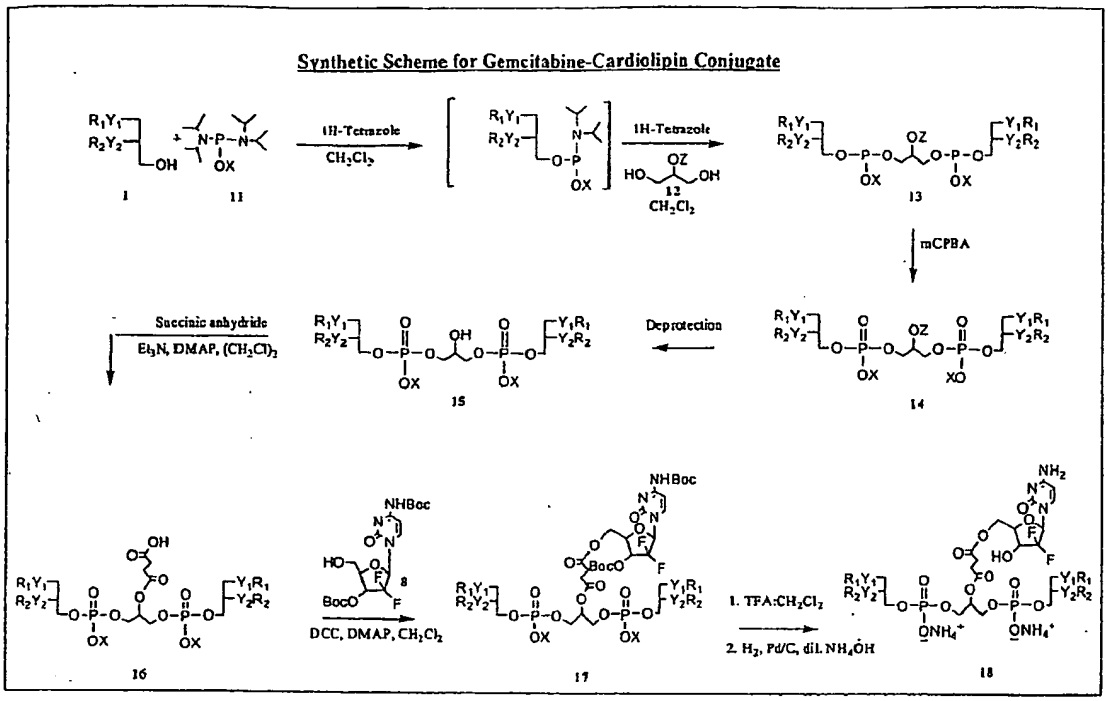

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
1H-Tetrazole
CH2Cl2
1H-Tetrazole
CH2Cl2
mCPBA
Succinic anhydride
Et3N, DMAP, (CH2Cl)2
Deprotection
DCC, DMAP, CH2Cl2
1. TFA:CH2Cl2
2. H2, Pd/C, dil. NH4OH

Fig. 3

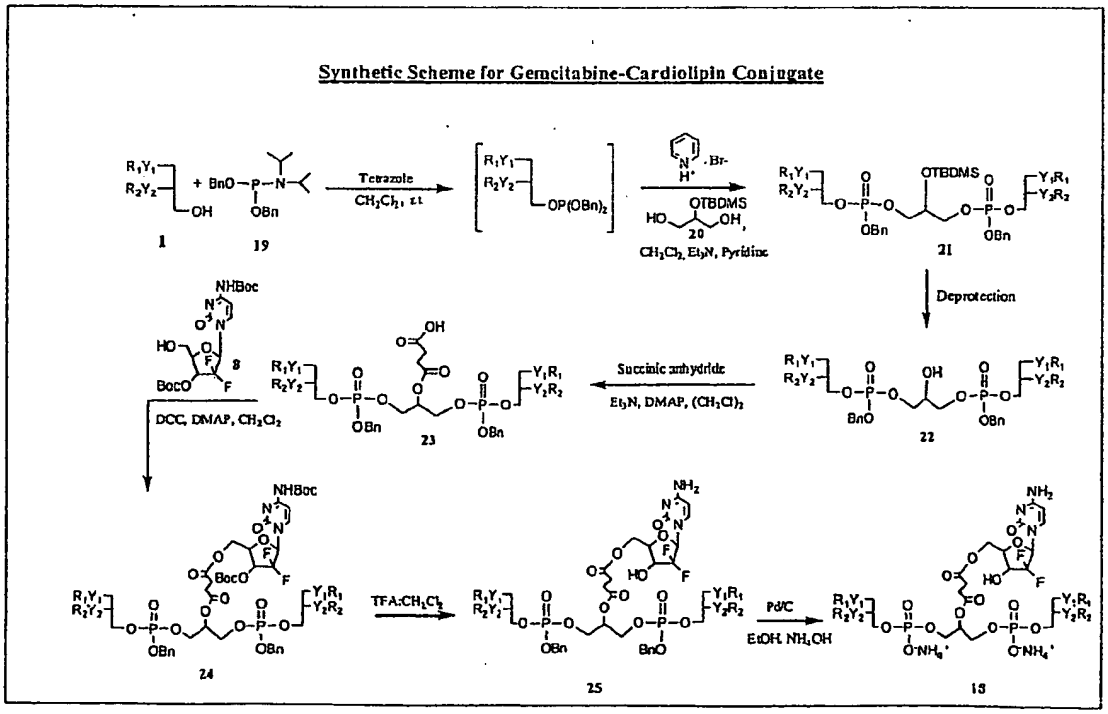

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
Tetrazole
CH2Cl2, rt
CH2Cl2, Et3N, Pyridine
Deprotection
Succinic anhydride
Et3N, DMAP, (CH2Cl)2
DCC, DMAP, CH2Cl2
TFA:CH2Cl2
Pd/C
EtOH, NH4OH

Fig. 4

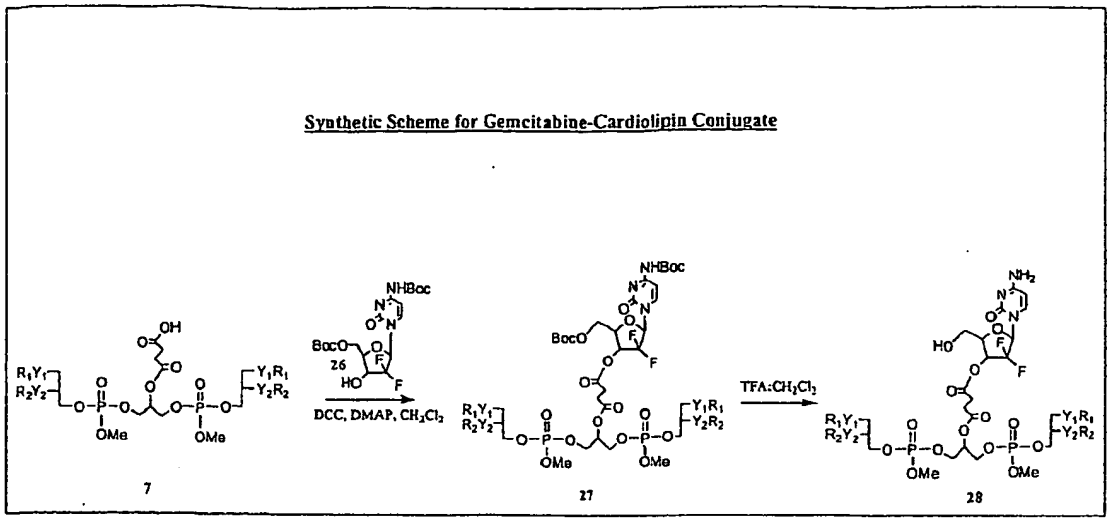

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
DCC, DMAP, CH2Cl2
TFA:CH2Cl2
7
27
28

Fig. 5

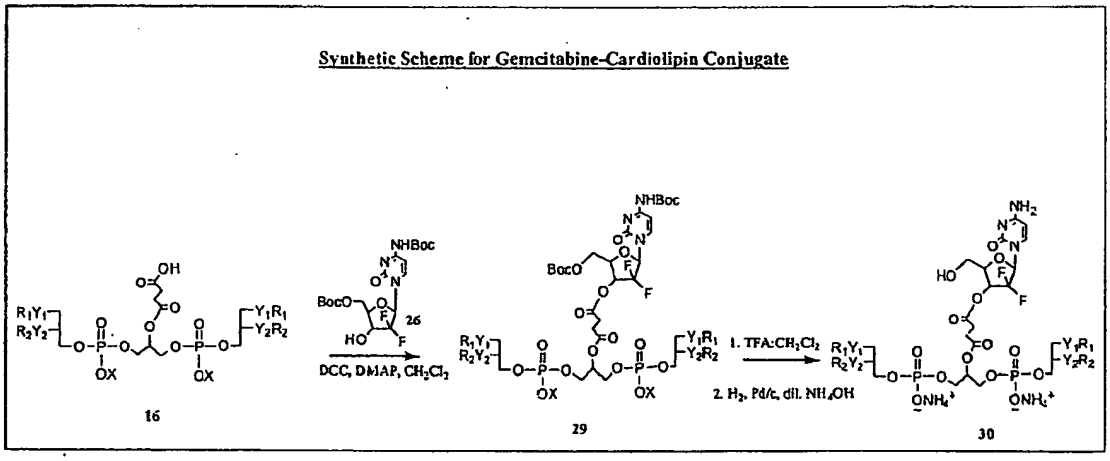

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
DCC, DMAP, CH2Cl2
1. TFA:CH2Cl2
2. H2, Pd/c, dil. NH4OH
16
29
30

Fig. 6

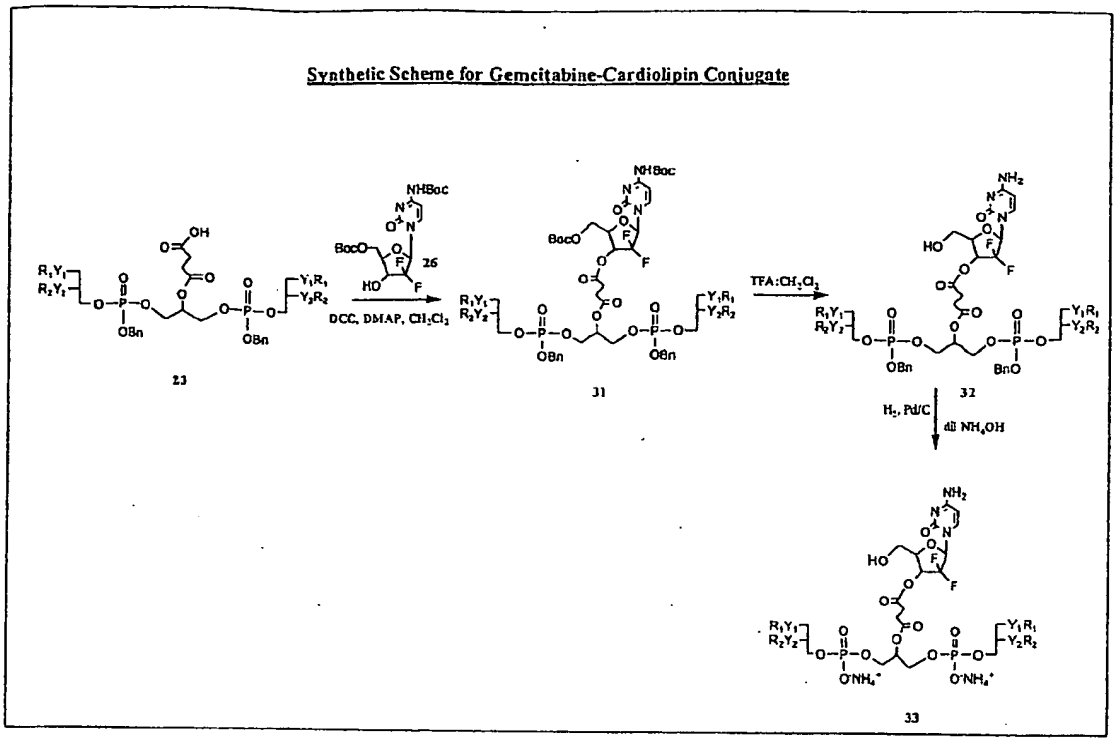

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
DCC, DMAP, CH2Cl2
TFA:CH2Cl2
H2, Pd/C
dil NH4OH
23
31
32
33

Fig. 7

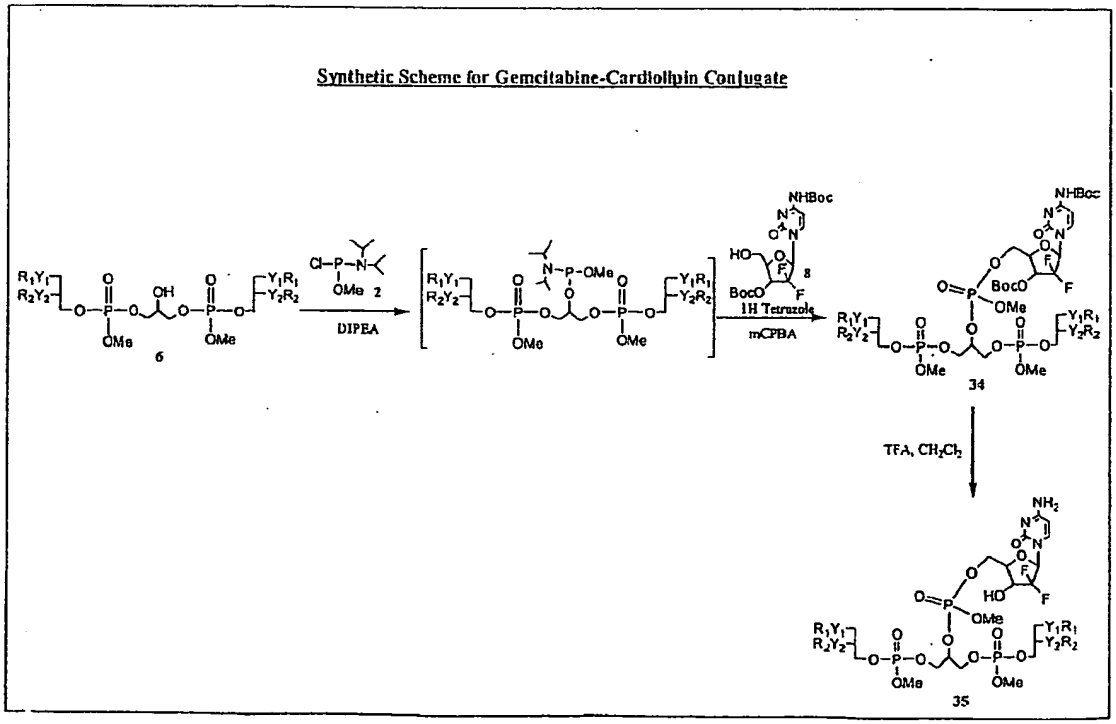

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
DIPEA
1H Tetrazole
mCPBA
TFA, CH2Cl2
6
34
35

Fig. 8

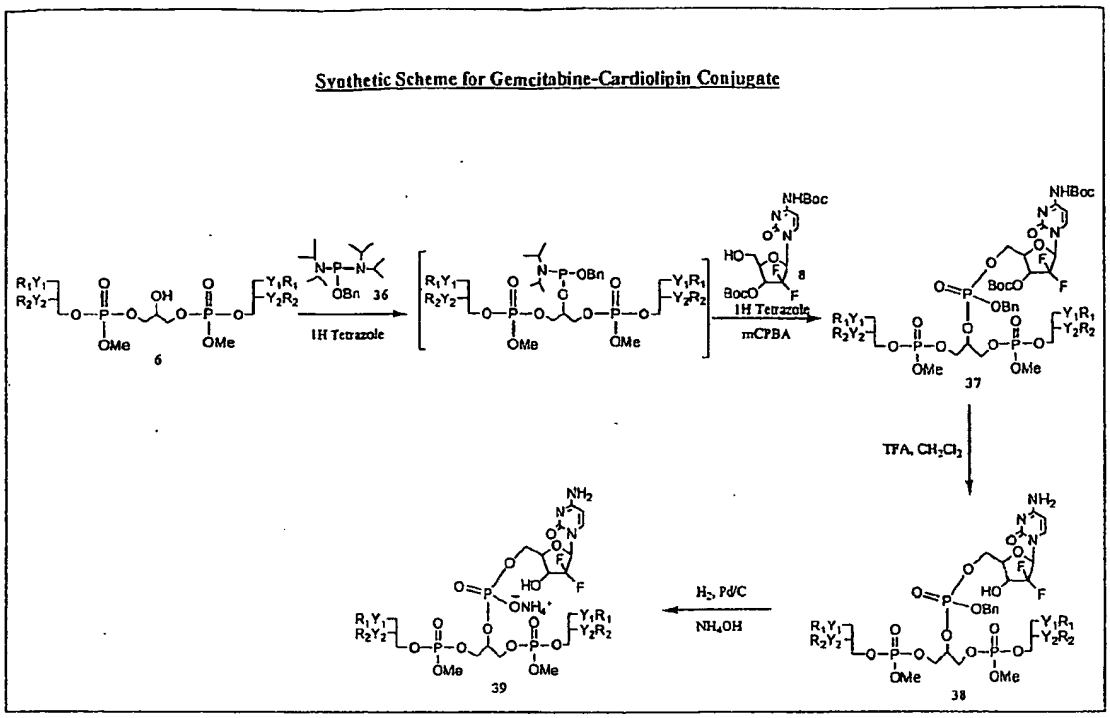

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
1H Tetrazole
mCPBA
TFA, CH2Cl2
H2, Pd/C
NH4OH

Fig. 9

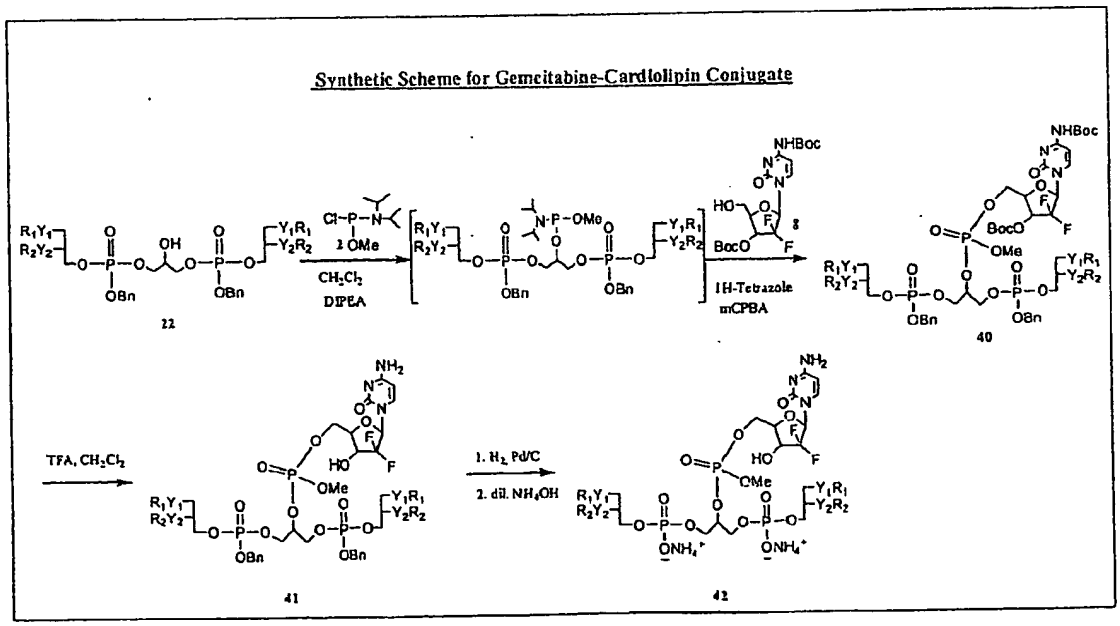

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
CH2Cl2
DIPEA
1H-Tetrazole
mCPBA
TFA, CH2Cl2
1. H2, Pd/C
2. dil. NH4OH

Fig. 10

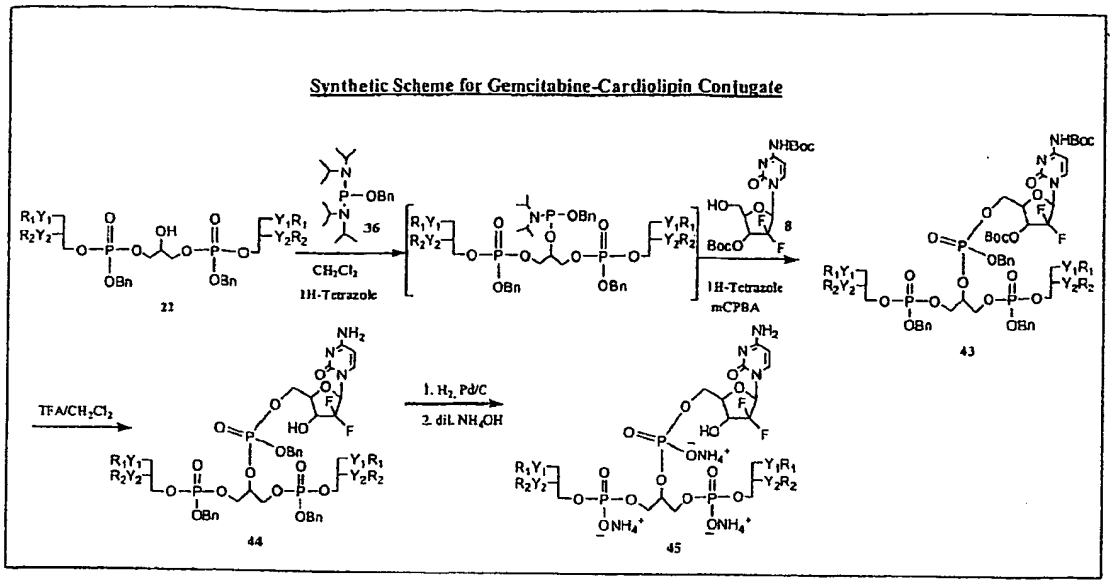

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
22
36
CH2Cl2
1H-Tetrazole
8
1H-Tetrazole
mCPBA
43
TFA/CH2Cl2
44
1. H2, Pd/C
2. diL NH4OH
45

Fig. 11

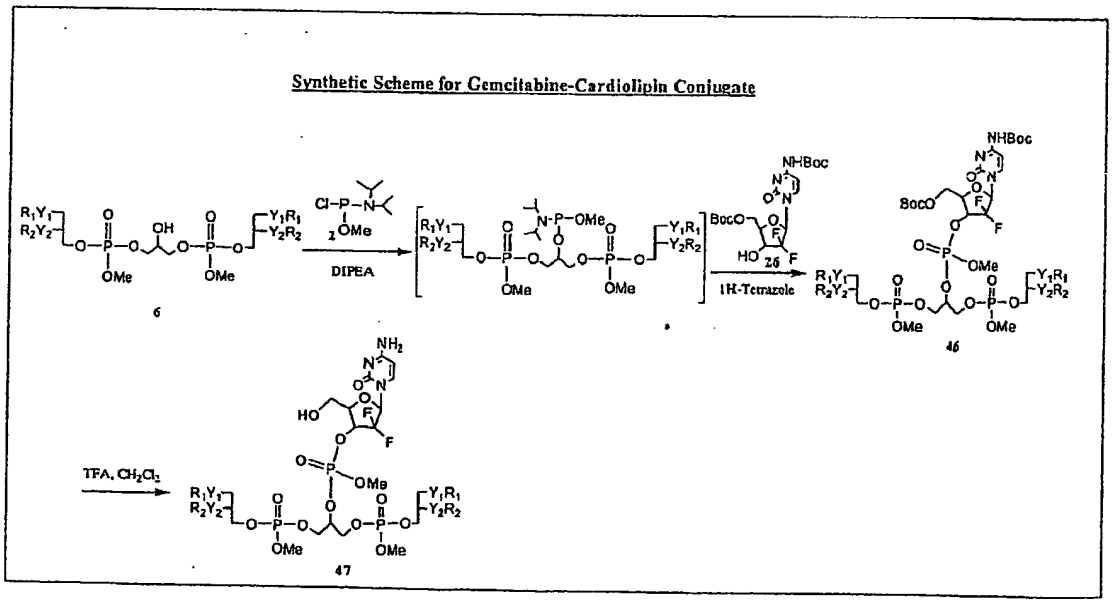

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
6
DIPEA
26
1H-Tetrazole
46
TFA, CH2Cl2
47

Fig. 12

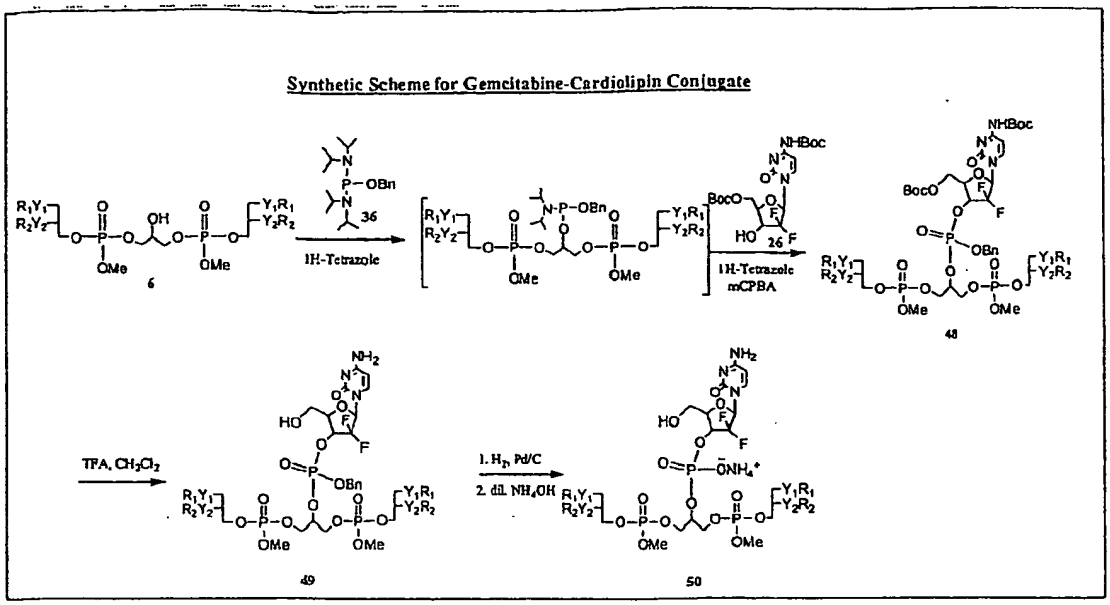

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
36
1H-Tetrazole
26
1H-Tetrazole
mCPBA
TFA, CH2Cl2
1. H2, Pd/C
2. dil. NH4OH
6
48
49
50

Fig. 13

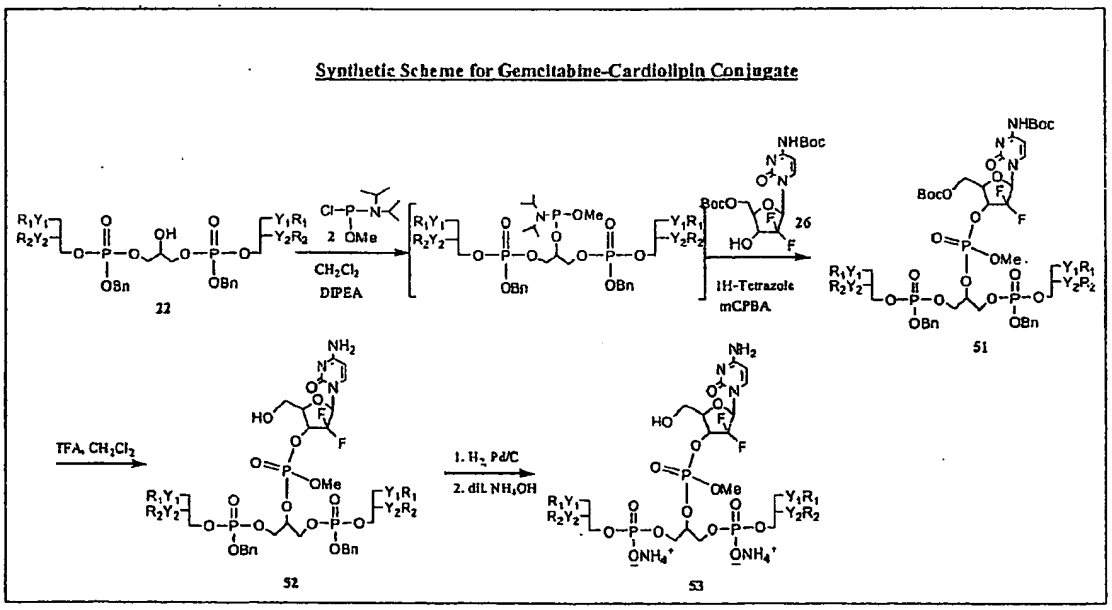

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
2
CH2Cl2
DIPEA
26
1H-Tetrazole
mCPBA
TFA, CH2Cl2
1. H2, Pd/C
2. dil. NH4OH
22
51
52
53

Fig. 14

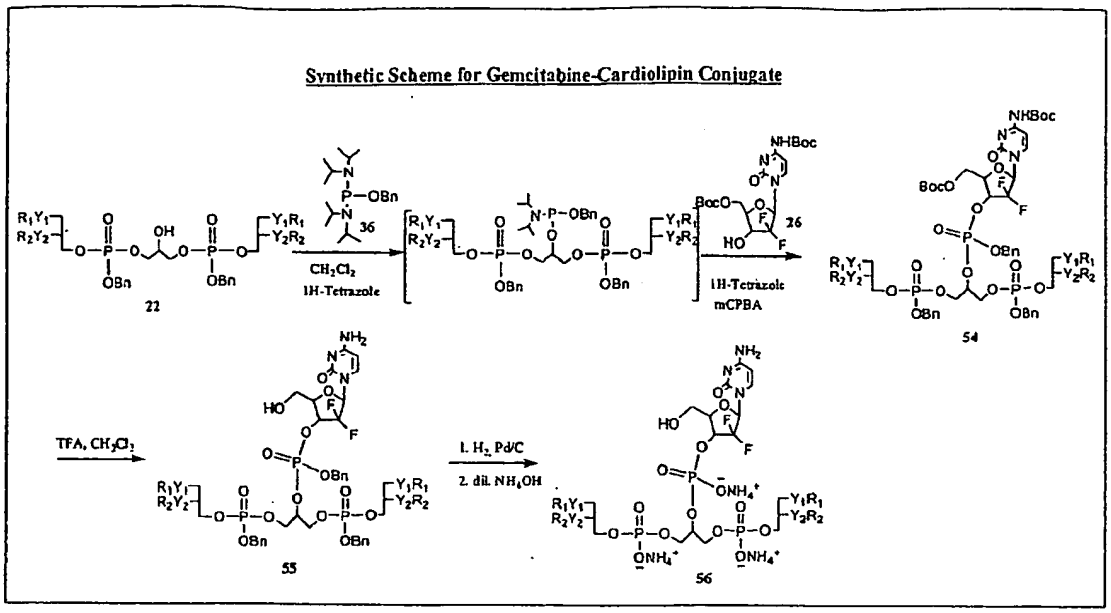

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
22
36
CH2Cl2
1H-Tetrazole
26
1H-Tetrazole
mCPBA
54
TFA, CH2Cl2
55
1. H2, Pd/C
2. dil. NH4OH
56

Fig. 15

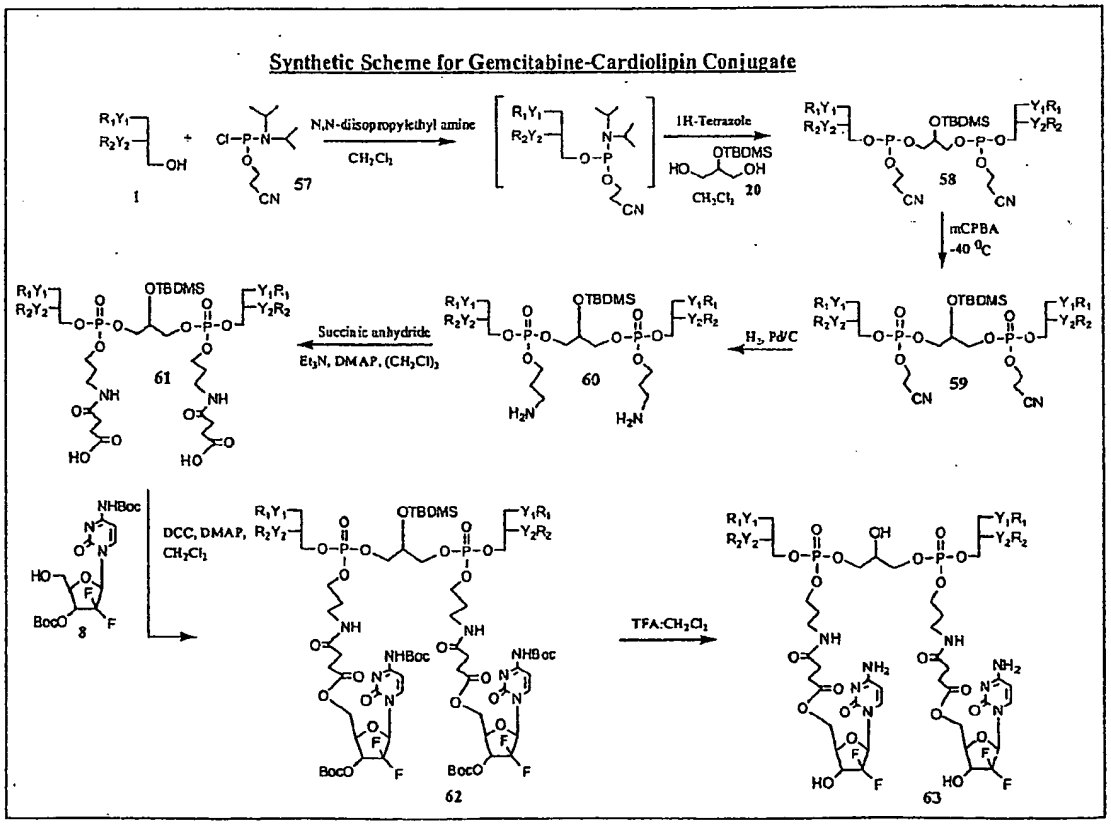

Synthetic Scheme for Gemcitabine-Cardiolipin Conjugate
1
57
N,N-diisopropylethyl amine
CH2Cl2
1H-Tetrazole
20
CH2Cl2
58
mCPBA
-40 °C
59
H2, Pd/C
60
Succinic anhydride
Et3N, DMAP, (CH2Cl)2
61
8
DCC, DMAP,
CH2Cl2
62
TFA:CH2Cl2
63

Fig. 16

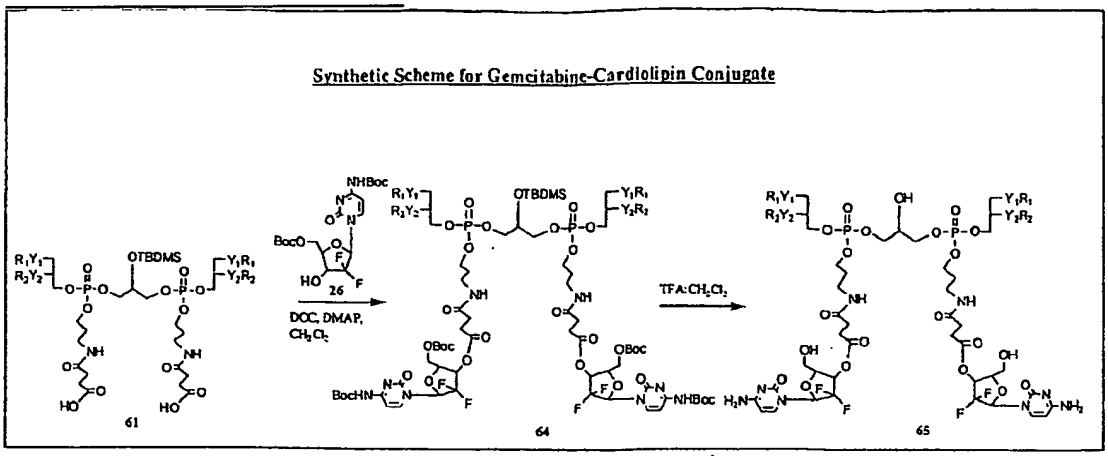
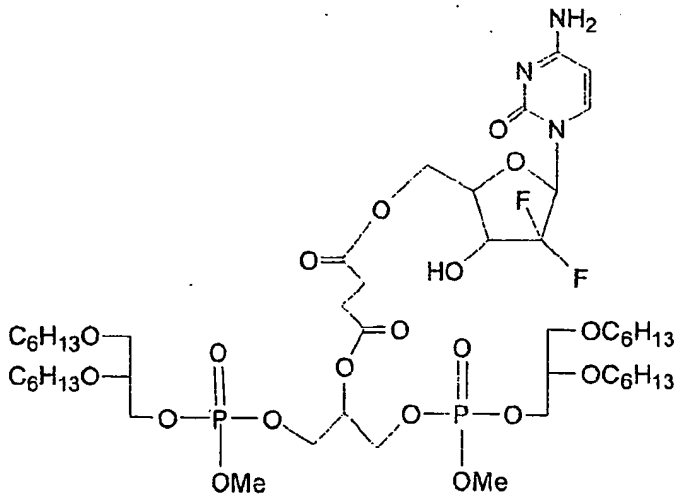


Fig. 17



XXXXIII

Fig. 18

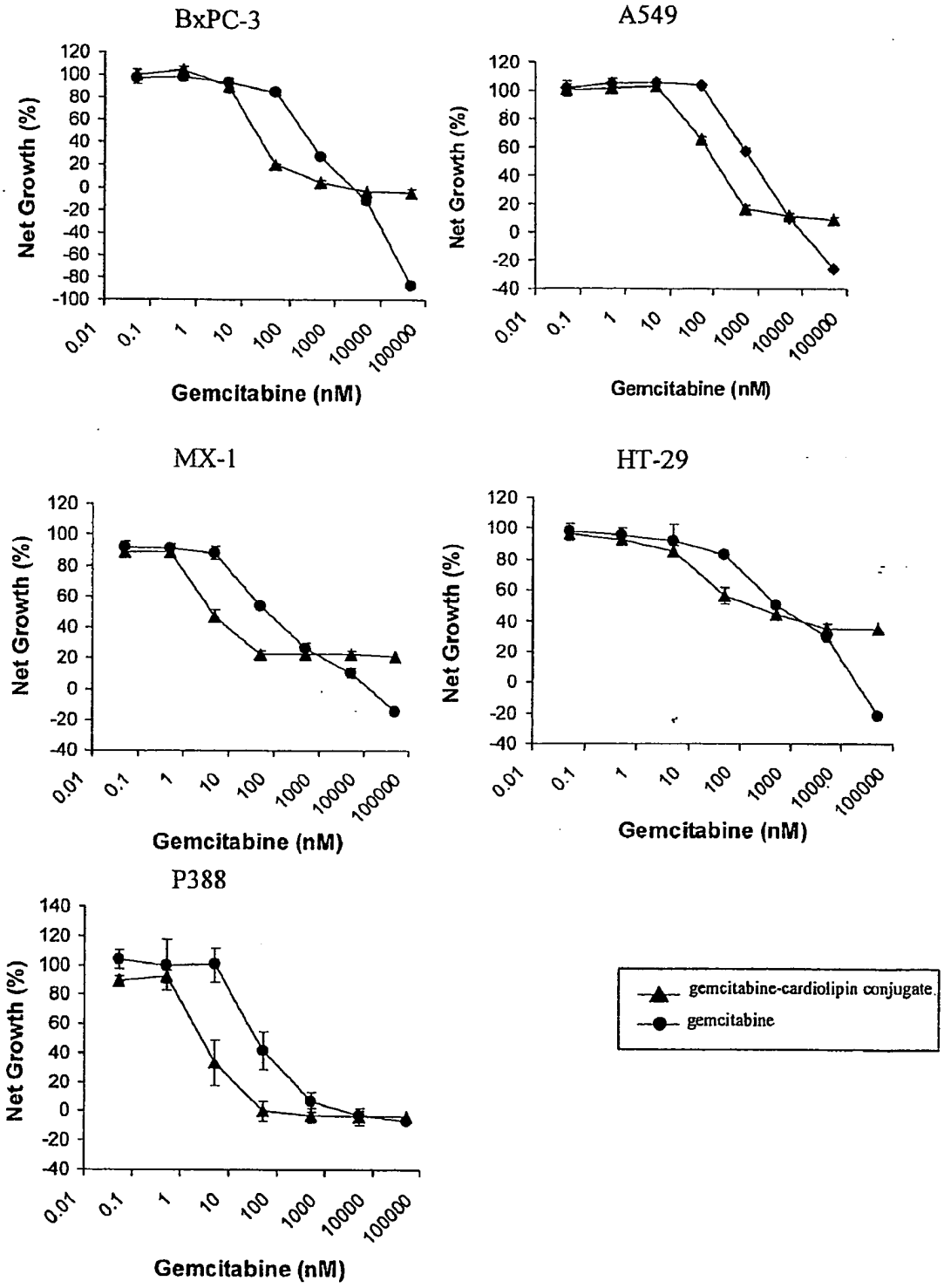

BxPC-3
A549
MX-1
HT-29
P388
Net Growth (%)
Gemcitabine (nM)
0.01
0.1
1
10
100
1000
10000
100000
gemcitabine-cardiolipin conjugate
gemcitabine

Fig. 19(a)

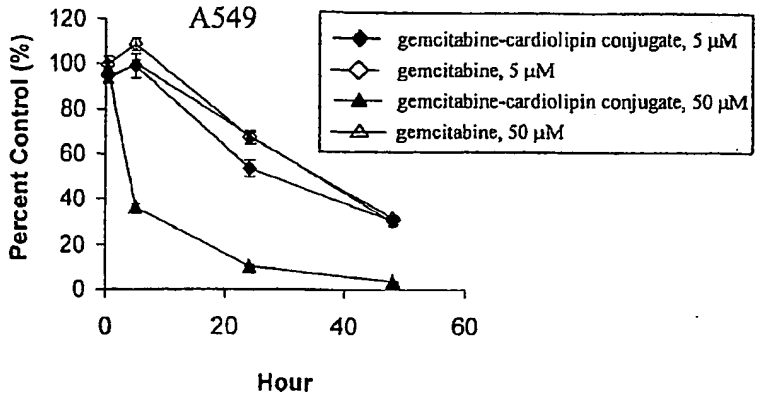


Fig. 19(b)

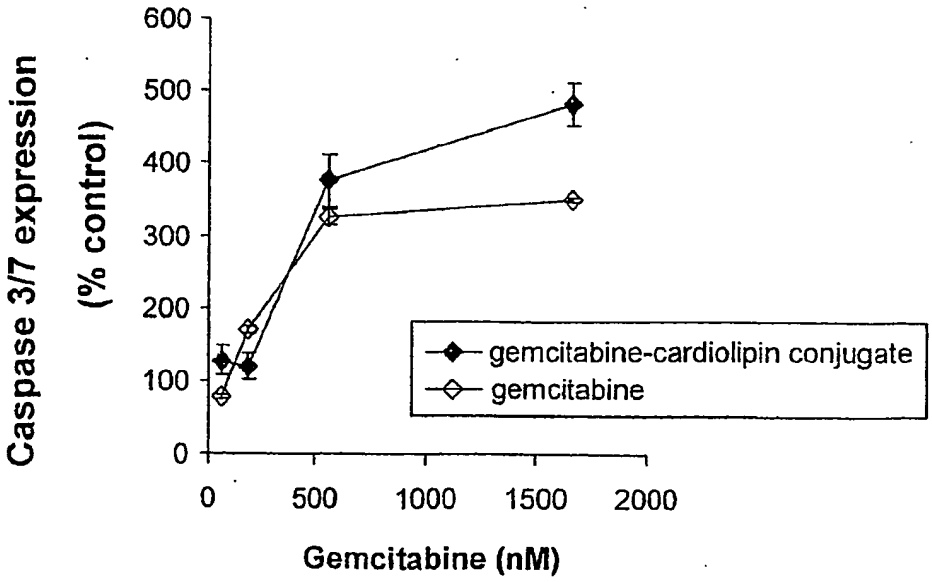
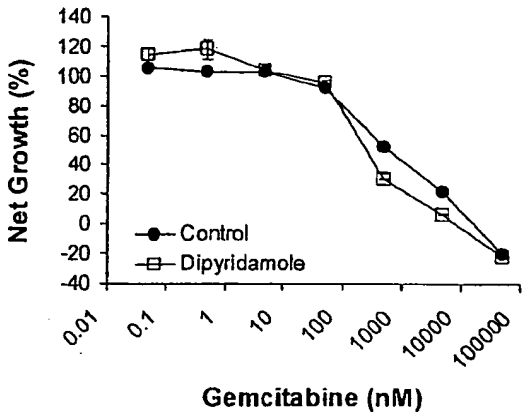


Fig. 20

a


Net Growth (%)
140
120
100
80
60
40
20
0
-20
-40
Control
Dipyridamole
0.01
0.1
1
10
100
1000
10000
100000
Gemcitabine (nM)

b

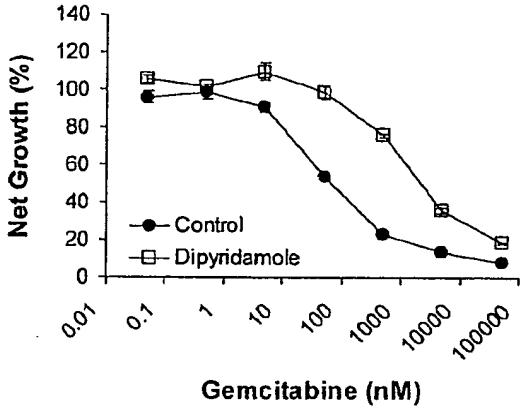

Net Growth (%)
140
120
100
80
60
40
20
0
Control
Dipyridamole
0.01
0.1
1
10
100
1000
10000
100000
Gemcitabine (nM)

Fig. 21

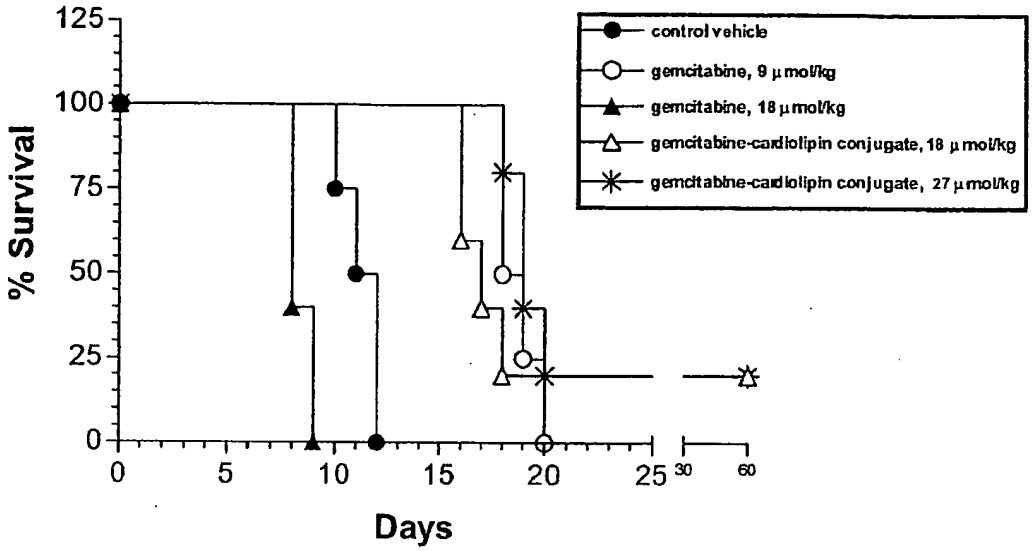

125
100
75
50
25
0
% Survival
0
5
10
15
20
25
30
60
Days
control vehicle
gemcitabine, 9 µmol/kg
gemcitabine, 18 µmol/kg
gemcitabine-cardiolipin conjugate, 18 µmol/kg
gemcitabine-cardiolipin conjugate, 27 µmol/kg

Fig. 22

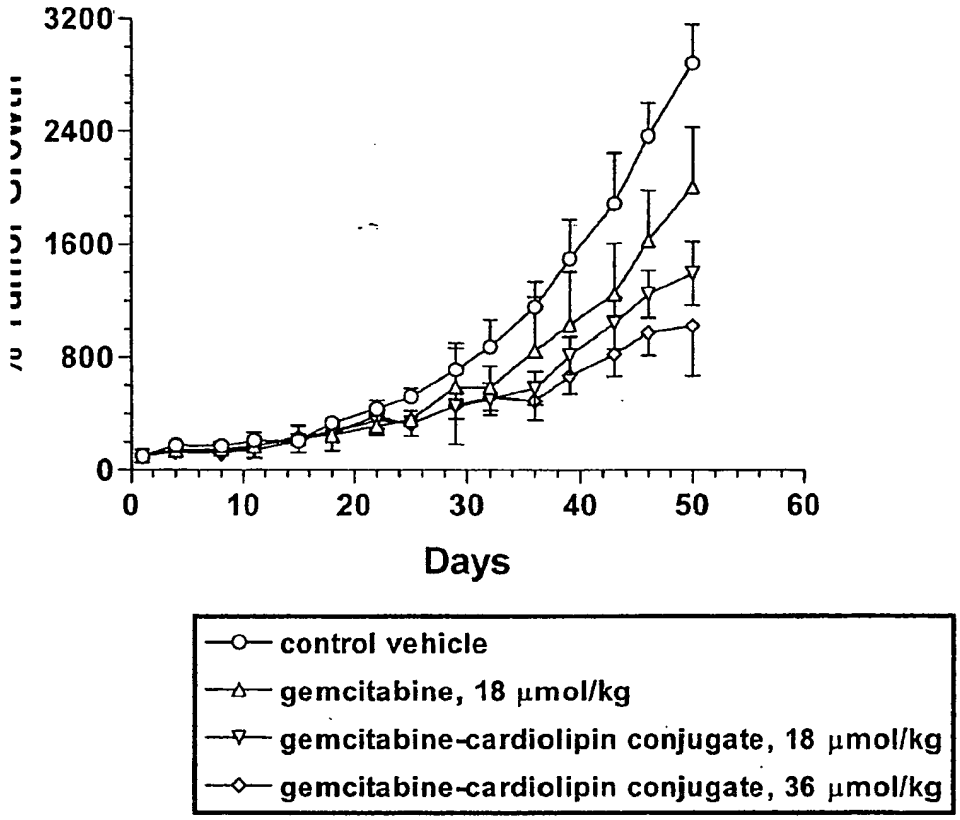

3200
2400
1600
800
0
0
10
20
30
40
50
60
Days
control vehicle
gemcitabine, 18 μmol/kg
gemcitabine-cardiolipin conjugate, 18 μmol/kg
gemcitabine-cardiolipin conjugate, 36 μmol/kg

Fig. 23